



February 24, 1977

Human rights and the scientist

'SCHOLARS of all kinds continue to be oppressed and persecuted in many parts of our contemporary world, and concerted attempts to impede their enquiries and suppress their conclusions are as common today as at any time in the past'. So begins a newly published report *Scholarly Freedom and Human Rights* (Barry Rose Publishers Ltd, £2.25) by a study group of the Council for Science and Society in collaboration with the British Institute for Human Rights. No documentation is given for the statement; indeed, the group has deliberately avoided quoting examples, but anyone with a mite of political awareness will know exactly what they are talking about and also that oppression is not restricted to any particular part of the world.

The central theses of the report are two: that there is a substantial body of international human rights law which should be adequate to cope with the sort of violations that are depressingly familiar; and that scientists, depending so much on communication, are peculiarly prone to what may seem to others to be minor constraints or irritations. Isolate an accountant from the literature and from his colleagues for a few years and afterwards he could soon pick up the threads of his profession; do the same to a scientist and his whole career may be ruined and others' impoverished.

The human rights legislation that has accumulated since the 1948 United Nations Universal Declaration of Human Rights is extensive, and the report provides a valuable guide. Of central interest to scientists are assertions such as that of the United Nations Declaration: 'Everyone has the right to freedom of opinion and expression; this right includes freedom to hold opinions without interference, and to seek, receive and impart information and ideas through any media and regardless of frontiers.' The problems, however, arise in the qualifications. The United Nations Declaration is only proclaimed as a 'common standard of achievement'—highly desirable but not legally binding. A document which binds its members and which provides a mechanism for redress—the European Convention on Human Rights—says almost the same thing, but it then goes on to add 'the exercise of these freedoms . . . may be subject to [legal and democratic] restrictions . . . in the interests of national security, territorial integrity or public safety, for the prevention of disorder or crime, for the protection of the reputation or rights of others, for preventing the disclosure of information received in confidence, or for maintaining the authority and impartiality of the judiciary'.

Some of these restrictions, the report points out, do no more than prevent anyone who does things like shout 'Fire!' in a crowded theatre from avoiding the legal consequences by claiming freedom of expression. But there are plenty of murky areas connected with 'national security', 'protection of morals' and so on, and it is a disappointment that the study group, having noted these areas, did not pause at such a point and devote rather more than three paragraphs to a discussion of the subject. Also, having drawn attention to differences in priorities between countries operating different economic systems, the group could well have elaborated rather more on the problems of misunderstanding between countries which rate civil rights high but give less guarantee of economic benefits and those which have an opposite order of priorities. There is one paragraph in the report in which it suddenly seems possible that this question will be analysed in depth, but the opportunity is passed over.

But what should scientists do when they hear of violations of human rights? We have not just an internationally accepted right to protest, says the report, but a duty to do so, and in a public way both individually and through organisations such as learned societies. Private persuasion is allowed its place but the major sanction 'must always be that of public protest'. The clear intention of the report thus emerges in its last few pages—bodies such as the Royal Society have no business steering clear of public pronouncements on the oppression of scientists.

Not for the first time, however, the Council for Science and Society has almost predetermined the conclusions of a study group by its choice of membership of that group. We greatly admire the vigour of John Ziman, convener of the group, and the integrity of its members—though it hardly seemed necessary for them to write that they all 'share a high degree of commitment to the ideal of the relentless and objective search for knowledge, wherever it may lead'. But was the outcome ever in doubt? Professor Ziman's views are well known on the subject of Royal Society intervention, and this makes the report's conclusions seem less weighty than they would have been had the Council gone to someone less committed to convene the group. The excellence of the sections on human rights law and on the needs of scientists remains undiminished. They should be widely read, and the hope must be that the document will have some effect. But its conclusions, put together a little skimpily as if they were self-evident, lack the corresponding weight. □



Peace research: the whys and the wherefores

Frank Barnaby, head of the Stockholm International Peace Research Institute (SIPRI), outlines the course being followed by military technology and offers his view on the role of peace research

"WITH the continuing technical progress and the current world-political constellation, it is highly probable that there will be an atomic world war before the end of this century." This statement was recently made by Professor Carl Friedrich von Weizsäcker, the West German physicist-philosopher, Director of the Max-Planck Institute in Munich, and author of the widely-discussed book *The Relevance of Science*. According to Professor von Weizsäcker a nuclear world war will probably be followed by the establishment of a world government which will ensure that no more nuclear world wars occur. In his view, then, it is likely to be the Third World War which will end all world wars.

Most students of military technology would agree that the probability of a general nuclear war is increasing—at least if only the consequences of advances in and the spread of military technology are taken into account. Three specific factors contribute to this conclusion. First, the nuclear arms race between the USA and the USSR could (and, therefore, probably will) eventually lead to a first-strike capability. This does not necessarily mean that one side will be able to destroy completely the other's capability to inflict casualties and damage in retaliation for a first-strike, but rather that this capability may be perceived to be sufficiently reduced to limit the casualties and damage to a level that might be considered acceptable for a given political goal. This level will depend on the recklessness and adventurousness of the political and military leaders in power at the time.

First-strike strategic nuclear weapons are likely to result from the improvement in accuracy of ballistic missiles. The accuracy of the current US Minuteman III land-based intercontinental ballistic missile, for example, is about 400 metres, at a range of about 13,000 kilometres. By the mid-1980s warheads with accuracies of about 30 metres may be available which, by any standards, will be first-strike weapons, even against small hardened targets. When it is remembered that the German V-2 missile could barely be depended on to hit London, the rate of progress which has been and which is still being made in missile technology will be realised. And similar progress has, of course, been made in many

other areas of military technology.

If the limit has nearly been reached in the performance of strategic offensive missiles, there is still some way to go in strategic defensive systems. Much research is under way in defensive weapons against ballistic missiles and in anti-submarine warfare. A breakthrough in these areas, which can by no means be discounted, would be extremely dangerous for world security.

When the political and military leaders perceive a first-strike capability to be near they may intensify measures to defend the population, and to protect industry and vital plants from nuclear explosions. The effectiveness of these measures will of course be taken into account when assessing first-strike capabilities. That perceptions about "winning" a strategic nuclear war are likely to be proved wrong, if ever put to the test, is hardly a consolation, since civilisation as we know it will be destroyed in the process.

The second reason for concern is the worldwide spread of the capability to produce nuclear weapons, through the spread of peaceful nuclear technology, and of the technical knowledge and expertise required for nuclear-weapon production. It is extremely unlikely that any new international measures to establish a viable non-proliferation regime will succeed where the Non-Proliferation Treaty has failed. The most that can be hoped for is that measures—such as moratoria on the reprocessing of reactor fuel elements to remove the plutonium from them and on the large-scale construction of breeder reactors—will be taken to slow down the rate of proliferation.

The Achilles heel of the proliferation problem is that sufficient fissile material for a modest nuclear force could be obtained, by virtually any country wishing to do so, on a small scale, and secretly. The components for a small reactor able to produce enough plutonium for say two atomic bombs per year are available on the open market. And the reprocessing of a few tens of kilograms of plutonium annually could be done on a laboratory scale. The indigenous clandestine construction of a small uranium enrichment plant is also within the capabilities of many countries.

It is already hard to know for certain whether or not some countries have, or are producing, nuclear weapons (Israel,

for example, has kept the world guessing for years), and this uncertainty will increase with time.

The third factor is the international trade in arms which is spreading the most sophisticated conventional arms worldwide. Since the October 1973 Middle East war this trade has increased at an unprecedented rate and is now virtually out of control. As a consequence, the arsenals of some Third World countries are more up-to-date than are those of some industrialised countries. Many of the aircraft and missiles supplied to the Third World are capable of delivering nuclear weapons (on today's standards, even a crude nuclear weapon with an explosive power equivalent to that of 20,000 tons of TNT should weigh less than 1,000 kg).

Some of the major recipients of arms become strongly bound to the supplier state, often to an extent hardly distinguishable from that of a close ally. This dependence is considerably heightened by the knowledge that in modern war munitions—particularly anti-tank and anti-aircraft missiles—are normally used at a very high rate. Victory may depend on the willingness of a great power to provide the massive quantities of replacements needed throughout the war. In this sense, the great power often becomes, implicitly or explicitly, the guarantor of the client state.

A war in, for example, an unstable region, involving one or more of these client states, could escalate from a conventional war to a limited nuclear war fought with the nuclear weapons of the local powers and then to the involvement of the great powers, committed to defend their clients—an involvement which might end in a general nuclear war. This end result is, of course, most likely to occur if one of the great powers perceives the chance of making a successful first-strike.

While not disputing the dangers inherent in unrestrained military technology, there are those who argue that increasing cooperation between states—in, for example, economic affairs—will prevent the great powers from pursuing policies which lead to a general nuclear war. Whether or not tendencies for cooperation will, in the next two or three decades, outweigh those for confrontation is a matter of personal judgment. I find it hard to be optimistic given the rapid rate at which military technology advances and the relatively slow rate at which effective bonds between nation states are formed.

Taken together, the three factors

will produce an extremely dangerous period in the not too distant future, unless steps are taken to prevent it. If, for example, a first-strike capability is developed it will take a very strong-willed and responsible leadership to resist arguments for its use. With so much at stake for us all, can we really afford to sit back and hope that the right men will be in power at the crucial times?

There is no reason why the current rate of progress in military research and development, the activity mainly responsible for progress in military technology, should not be sustained. In fact, because R&D is the most difficult of all military activities to control, one can expect the rate of progress to continue to increase. Military technology has indeed come a very long way since the Second World War. Where will this technology take us by the end of this century? Scientists who ponder this question are generally doomsayers.

All of the ramifications of advances in military technology are known only to an extremely small group of people outside governments—to a few hundred scientists worldwide. It is a sobering thought that even within the world's governments at most a small handful of political leaders and their advisers are aware of the consequences of the onward rush of military technology. Under these circumstances and given the catastrophic nature of nuclear war, are the few scientists who do know the facts under a special obligation to publicise them?

The social responsibility of scientists has been much discussed over the past twenty years but inconclusively. There does, though, seem to be wide agreement that scientists do at least have a duty to inform non-specialists about any scientific developments (or the application of research results) which could lead to adverse social consequences. And the more dire the consequences then presumably the greater the duty to inform. But to whom should the information be addressed? In what form should it be presented? And how can its dissemination be best achieved?

These questions are particularly relevant to the Stockholm International Peace Research Institute (SIPRI), an independent institute established in 1966 and financed by the Swedish Parliament, which publishes factual studies on military technology, armaments, and disarmament (see *Nature*, **246**, 397-400; 1973). During SIPRI's lifetime the situation in armaments and disarmament has changed considerably. Ten years ago there was still reason to hope that arms control negotiations would succeed in stopping the Soviet-American arms race and lead, step-by-step, to significant disarmament. We

now know differently. Throughout this period, the arms race has continued virtually unconstrained and the only weapons destroyed by international agreement have been biological weapons — weapons of little military interest.

Arms control may lead to the management of the arms race. Arms control may contribute to Soviet-American détente. But, other than the actual negotiators and committed political leaders who soon develop a high level of 'professional optimism', few now believe that the arms control approach will actually lead to disarmament.

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In my opinion, this lack of success, and the resultant ever-increasing probability of nuclear war, demands urgent action. Whereas in the mid-1960s it seemed that SIPRI's research should be aimed at the specialists advising those decision-makers and diplomats able to affect arms control negotiations, it is now clear that a much wider audience must be reached.

The political leaders of the great powers clearly lack the will to negotiate nuclear disarmament. There are two possible ways of applying the necessary pressure on these reluctant politicians. One is through public opinion, or at least the most influential sections of it, and the other is concerted action by the smaller countries.

SIPRI has neither the skills nor the resources needed to appeal directly to the public. But it can, and does, provide information in a form easily used by those who have — particularly

journalists working for the press, radio and TV. SIPRI also makes sure that its material reaches the relevant authorities in the smaller powers.

A few of these powers—Canada, Mexico, the Netherlands, Sweden and Yugoslavia, among others—have a sizeable group of officials well trained in disarmament and related matters. But the bulk of the smaller countries do not. The one or two individuals in these countries dealing with the subject need information in a summarised and easily digested form which should also give a sense of the urgency of the current situation. An authoritative institute is able, and most would say has the duty, to provide this crucial service in addition to its various research publications.

All of this pre-supposes that far-reaching disarmament (beginning with nuclear disarmament) can be directly negotiated in today's world of 150 or so states using the traditional methods of diplomacy and international law. SIPRI believes this to be so. SIPRI also believes that unless this process is soon begun it may be too late to prevent a nuclear world war.

Some peace researchers, however, reject these beliefs. They argue instead that conflict—leading eventually to general nuclear war—is inevitable because of the very nature of the present international system. The only way to prevent this, they say, is to change totally the system by developing entirely new international political and social institutions. In their view it is unrealistic to expect today's world to disarm, and even if it did the likelihood of nuclear war would remain. In my opinion, this approach is not only incorrect but dangerous. There is probably insufficient time to produce a new world order, even if such a thing were feasible. And there is the danger that too much emphasis on the inevitability of nuclear war may make it a self-fulfilling prophecy.

In summary, I believe that the dissemination of objective information on armaments and disarmament to specialists is still crucial but it is not enough. In addition, it is necessary to explain to non-specialists where military technology is taking us—particularly to the groups mentioned above—in a way which will stimulate their interest and hopefully provoke them to action. In this sense, SIPRI's work has become somewhat more 'political'.

The fear that the presentation of material in a brief and readable (even somewhat enlivened) form must damage the reputation for objectivity of a research institute is, in my opinion, unfounded. When the need for political effectivity is urgent and great it is even defeatist. □

Czechoslovakia (1)

The general . . .

A Special Correspondent discusses the climate in which science operates in Czechoslovakia

THE fact that by comparison with Czechoslovakia probably no other state in the world has so many members of its political leadership with the highest academic qualifications seems to express a very attractive idea—namely, that educational opportunity is equal for everyone, that political leadership emerges in a democratic way and that people make rational choices for the best of the society. The logical conclusion that those who take decisions are the most competent is undermined by two factors.

First, in practice it is the Communist Party which is the repository of power. For example, only those scientists who conform to party rulings are acknowledged as scientists. And the acquisition of school qualifications or of a university degree and high academic honours in the space of months may well accompany a person's improving political fortunes. Still, the fact that political leaders regard it as worthwhile to present themselves as academics symbolises the significance which they attribute to the community of learning.

Secondly, some prominent intellectuals in Czechoslovakia are now in revolt against the present regime. The *Charter 77* human rights manifesto, originally signed by more than 200 persons of "various shades of opinions, faiths, and professions", included some 25 scientists and doctors. It has since been countersigned by at least another 200 supporters, and contains a number of accusations of relevance to all who value academic and scientific freedom (see box).

The current Five Year Plan (1976–80) shows clearly that the present leadership continues to hope for an important contribution of science to economic development. Expenditure for research and development is to grow by a third of the present sum to 3.8% of GNP in 1980, and the proportion of people working in science and technology is to be 21 out of every thousand working in general. Expenditure on equipment has nearly doubled in the past five years and is to grow still further; so will the salaries of scientists.

The significance attributed to science by the present regime in Czechoslovakia is not just theoretical. Yet the apparently grave difficulties achieving the expected results from

the effort put into science seem more and more clearly to arise from the problems inherent to the system itself and not just from the capacities of its leadership.

Of the two different views about the preconditions for the advancement of learning—one stressing free inquiry, the importance of competition and the natural selection of ideas, the other maintaining that an attainment of shared norms and procedures by the scientific community is desirable and that scientific effort as a whole can and should be rationalised—communist ideology is decisively in favour of the second view, since it believes that the development of science can be encouraged when planned. Virtually the whole of scientific effort is subject to state planning in Czechoslovakia.

Even though science is administratively subordinate to the central agencies of government, it would be misleading to say that one identifiable centre prescribes what should be done. Short-term plans spell out work assignments for practically every scientist; this is done on the basis of two-yearly surveys of the country's material and manpower resources in science. The latest of the regular five-year plans was worked out by 800 scientists and specialists from production, directors and representatives of

the relevant working places and, of course, government ministries. Political supervision comes only through the political bounds on the participants themselves. Occasionally mention is made of preparations for a long-term plan, but so far nothing has materialised.

One of the basic difficulties is the right balance between research and development assignments. The current five year plan for Czechoslovak science foresees 346 main tasks; 70% of these are oriented towards current technical developments, 23% are concerned with other social needs, such as medicine and social science, and 7% are tasks of pure research. The proportion of uncommitted to committed goals is, even in the country itself, considered low.

One problem frequently pointed out in Czechoslovakia is the highly unnatural age-structure of people working in research. The official figure for the average age is 45, but this does not express the fact that there is a whole generation missing. After the continual purges in the early 1970s and the emigration of many highly qualified scientists to the West, the number of people between 30 and 50 diminished drastically. There are institutes where only isolated persons survived the past few years. The zigzag course of Prague's present policy has meant alternating periods of relative reconciliation and dogmatic reassertion. Changes and deviations from the science plan require a very expansive, unproductive bureaucracy, and the administrative complex is very demanding of scientists' time—especially when taken together with permanent political courses and meetings.

'A virtual apartheid'

Some extracts from the *Charter 77* human rights manifesto:

The right to freedom of expression . . . is in our case purely illusory. Tens of thousands of our citizens are prevented from working in their own fields for the sole reason that they hold views differing from official ones, and are discriminated against and harassed in all kinds of ways by the authorities and public organisations. Deprived as they are of any means to defend themselves, they become victims of a virtual apartheid.

. . . countless young people are prevented from studying because of their own views or even their parents'. Innumerable citizens live in fear of their children's right to education being withdrawn, if they should even speak up in accordance with their convictions.

Any exercise of the right to 'seek, receive and impart information and ideas of all kinds, regardless of frontiers, either orally, in writing, or in print' . . . is followed by extra-judicial and even judicial sanctions . . .

No philosophical, political, or scientific view or artistic activity that departs ever so slightly from the narrow bounds of official ideology or aesthetics is allowed to be published.

Many scholars, writers, artists and others are penalised for having legally published, years ago, opinions which are condemned by those who hold political power today.

All national institutions and organisations are in effect subject to political directives from the machinery of the ruling party.

Clause 2, article 12 of the [International] covenant [on Civil and Political Rights] guaranteeing every citizen the right to leave the country is consistently violated or under the pretence of 'defence of national security' is subjected to various unjustifiable conditions . . . The granting of entry visas to foreigners is also treated arbitrarily, and many are unable to visit Czechoslovakia merely because of professional or personal contacts with those of our citizens who are subject to discrimination.

Czechoslovakia (2)

... and the particular

Vera Rich considers the position of scientists in the Slovak Republic

SOME Western analyses of the Soviet and East European interest in detente stress their need for modern technology. Insufficient modernisation of industrial production in these countries is said to be due to the difficulties in implementing new technologies. The problem may not be a simple mechanical one, though; it may also be related to the relegation of science to the status of a mere tool whose direction has been predetermined by the needs of society.

One of the main tenets of science policy throughout the Comecon block is the tightening of links between research and industry. The place of science in the economy is assessed primarily for its practical and immediate benefits. In the case of a relatively newly-developed area such as Slovakia, which for many years was the 'backward' partner of the Czechoslovak federal state, this can mean that scientists bear responsibility for deficits due largely to planning errors and past neglect.

Slovak research scientists have recently been blamed for deficiencies in the local metallurgical and engineering industries. This involves some 40% of all research workers in Slovakia, or more than 15,000 persons. According to the November 1976 meeting of the Central Committee of the Communist Party of Slovakia, "the slow introduction of new ideas in production and the contribution of research institutes so far are not satisfactory." There is a high turnover of leading technical and managerial personnel, weaknesses in planning and leadership, and a general failure to use modern equipment to the best of its ability.

Federal planning aims to "balance the sociopolitical and economic standards of the Czech and Slovak republics" and the development of Slovak industry is a major theoretical issue. The need for such a development is reflected in the targets for the present Five Year Plan: overall Czech industrial production is to be increased by 32.3% and Slovak production by over 44%. This means that output from Czech industrial enterprises must rise by 48.5% and from Slovak enterprises by 70%. The targets are a 50% rise in Slovak exports with engineering exports in particular rising by 100%. It is difficult to imagine that all these figures will be met, particularly

as Slovakia's main 'export' products—steel and aluminium—are not in fact exported in ingot form, but go to Bohemia to be manufactured into finished articles, which are then credited to the Czech Republic.

The slow growth of Slovak industry is partly historical in origin—when the Czechoslovak Republic was set up by the Treaty of Versailles, the Czech lands, which had been under Austrian rule, already had flourishing industries, while Slovakia, which had formed part of the kingdom of Hungary, was largely agricultural. The policy of the First Republic was to maintain this *status quo*—thereby causing considerable Slovak resentment against the federal government. During the late 1940s the industrialisation of Slovakia was a major issue. Students uncertain of which subject to pursue were urged into technology, in particular metallurgy.

One of the first actions of the new communist government was to start planning the vast East Slovak Iron Works at Košice. This was primarily a political decision: the only resources the area could offer were limestone for fluxing and a large labour force. Iron ore had to be brought from the Ukraine and coke from Czech Silesia. One of the charges brought against Slansky, the former Party Secretary, during his trial in 1952 was that this was an "impossible" scheme, and construction at the plant was halted for three years. In 1968, when open discussion became possible under the Dubček regime, it was revealed that steel produced at Košice was costing 8–10 times that produced in Japan, although Japan was shipping iron ore from Australia and Brazil and coke from Australia. Since 1968, the Husak government has invested sums in Slovak industry which are out of all proportion in comparison with the population of the republic, causing some resentment from the Czechs. The Slovaks, however, stress the need for even more investment. In September, 1975, Miloslav Hruskovic, the Secretary of the Central Committee of the Slovak Communist Party, noted that "serious problems persist in the capital investment plan", and that scientific and clinical development has not been "reflected in investment construction as much as it should have been" (no details were given).

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Camera Press

Gustav Husak, Communist party head

Improvements

Certainly investment has produced considerable improvements. During the 1971–75 period industrial production in West Slovakia (mainly chemical and engineering) is said to have risen by 54.6%, while in East Slovakia the "volume" of production increased by 66.2%. There was considerable investment in the chemical works at Bratislava, and the notorious Košice ironworks now has the second highest output of all Czechoslovak metallurgical plants, and the lowest manufacturing costs (no details given). And although the Federal reports indicate a general lack of enthusiasm and expertise in the use of computers, particularly for information and planning work, the development of a scientific and technical information system in Slovakia was, according to the Slovak Minister of Development and Technology, J. J. Busa, going forward well. The new arrangement, by which Cuba will supply the Comecon countries with nickel will undoubtedly benefit the nickel-works at Sereď, which until now has had to use ore from Albania.

Nevertheless, according to Hruskovic, what is needed is a greater intensification of production, together with a more economic use of raw materials, commodities and energy and an improvement in quality and technical standards. The Central Committee of the Slovak Communist Party proposes a stick-and-carrot policy for scientists and technologists: those who "have the courage to take risks on the challenging part of technological progress" will receive "moral rewards" and sharply-differentiated "material incentives" unhampered by "unwarranted egalitarianism, because this slows down progress". Those, however, who cannot "cope with the tasks of the present", should be transferred to other posts "tactfully, and before it is too late". □

BRAZIL

A growing resistance

Bruce Handler reports from Rio de Janeiro on recent developments involving the controversial German-Brazilian nuclear deal

ATTEMPTS by the Carter Administration in the United States to undermine the controversial nuclear technology agreement between West Germany and Brazil are resulting in precisely the opposite of what Washington wants. Brazil are resulting in precisely the go through with the atomic pact. Formerly divergent internal currents in Brazil are rallying behind the Brazilian government in defence of the agreement with Germany. And other Latin American countries—especially Argentina, which has always been considered Brazil's principal rival—are sticking up for Brazil in an unusual display of Latin American solidarity.

Part of Jimmy Carter's election campaign platform consisted of promises to oppose the spread of nuclear technology, especially in the underdeveloped Third World. And just a few days after taking office, he dispatched his Vice President, Walter Mondale, to West Germany to ask Chancellor Helmut Schmidt to reconsider German plans, under the nuclear agreement, to aid Brazil in developing its own full-fuel cycle. This German knowhow is to allow Brazil to enrich its own uranium for use in electricity-producing nuclear reactors, but it also will make Brazil theoretically capable of producing plutonium—a key ingredient in nuclear weapons.

After some initial backtracking, however, Germany said it will go ahead with the Brazilian agreement as planned. Brazil declared flatly that it "sees no possibility" of altering or delaying the pact, as the United States wants. And Paulo Nogueira Batista, the head of the Brazilian government's nuclear power company, Nuclebras, told the press: "The agreement is being carried out on schedule, and in some cases you could say we are making even more progress than we expected."

Brazil's foreign minister, Antonio F. Azeredo da Silveira, did say later, though, that Brazil is willing to discuss "global aspects" of the problem of the spread of nuclear technology. This, the minister noted, ought to prove to President Carter that Brazil intends to act like a responsible, adult nation. Azeredo da Silveira invited the US Secretary of State, Cyrus Vance, to Brazil for talks, and it appears Vance will come.

The attempted pressure-play from Washington resulted in a remarkable outpouring of unified internal support for Brazil's authoritarian, military-run government, which had pressed hard to get the nuclear agreement with Germany in the first place. The actual agreement was signed in mid-1975, but the Brazilians had begun secretive negotiations with the Germans several years earlier.

Marcos Freire, a senator from the sole opposition political party permitted in Brazil, said: "The United States has no moral authority to interfere with the nuclear treaty." He speculated that "powerful economic interests" might be behind Washington's meddling, in view of the fact that the total value of contracts to be signed under the Brazilian-German agreement is conservatively estimated at \$5,000 million.

Another member of the opposition party, Representative Joao Cunha, said Brazilian President Ernesto Geisel, "speaking in the name of all Brazil's 110 million citizens, should repudiate this US manoeuvre."

Even Brazil's academic and scientific community, which under the current government tends to stay as far away from politics as possible, has spoken up with regard to the nuclear agreement. "This government is correct in not permitting outside interference in affairs over which it has exclusive sovereignty," said Professor Marcelo Dami, a physicist from Sao Paulo. Dami made it clear that he did not like the secretive way in which the government had gone about negotiating the agreement with the Germans and also that he has reservations about the type of fuel that was chosen for atomic reactors that are to be built in Brazil under the pact. "But disagreements of this nature now must be put in the background, in view of higher national interests that are at stake," he said.

Helio Jaguaribe, a political scientist whom some members of Brazil's military regime regard as a "dangerous intellectual," also came out in full support of the government. "President Geisel deserves the warmest applause from all Brazilians for the firm position he has taken in supporting the agreement," he declared. "President Carter's concern about the proliferation of nuclear weapons is fully valid. But Brazil has no nuclear weapons, and it does not intend to have them."

He said a distinction has to be made between stopping the proliferation of nuclear weapons and "the desire of the superpowers to maintain a monopoly

on nuclear technology." The Brazilian government, furthermore, was "absolutely right in not signing the Nuclear Non-Proliferation Treaty," since to do so "would perpetuate the underdevelopment of third world countries."

Unexpected defence for Brazil came from across the border in Argentina, which until now has been wary, to say the least, of its fast-developing neighbour's nuclear energy programmes and goals. Argentina began its own nuclear programme in the 1960s and was regarded as the most important country in Latin America in terms of atomic development until the German-Brazilian deal emerged.

"Argentina supports the Brazilian government in the nuclear field," declared the newspaper *La Opinion* of Buenos Aires, which is said to reflect the official line of President Jorge Videla's military-run regime. "The US offensive against Brazil, which is aimed at stopping progress on its plan with Germany to install eight more nuclear reactors, a uranium enrichment plant and a fuel reprocessing plant, is a blow which could be fatal to Brazil's nuclear industry. What happens to Brazil today could happen to Argentina tomorrow."

And in a statement that must have surprised a lot of Brazilians, *La Opinion* wrote: "Brazil is not just any developing country. It is a co-participant in the destinies of Argentina, in the Latin American context."

Amid the renewed debate surrounding the Brazilian-German nuclear pact, Brazilian government officials have hastened to repeat this country's justification for seeking the agreement in the first place:

- Brazil produces only 20% of the crude oil it needs every year. Its domestic coal is of low quality. Its remaining major potential hydroelectric sources are in the Amazon jungle, thousands of miles away from its centres of population and industry. Therefore, Brazil must develop nuclear energy as a source of electricity.

- Brazil must possess the knowhow for supplying itself with nuclear fuel. Despite a US offer to furnish already processed enriched uranium (with no transfer of technology) for Brazilian reactors, Brazil cannot run the risk of remaining dependent on foreign sources for energy. (Look what happened to the United States during the Arab oil boycott, a much heard Brazilian argument goes.)

- Brazil is a peaceful country. It doesn't need or want an atom bomb, and it would not divert resources from vital social and economic needs to build one. And besides, the safeguards in

the German-Brazilian nuclear agreement were approved by the International Atomic Energy Agency, with both the United States and the Soviet Union voting in favour.

Although none of the reactors or fuel processing equipment specified in the nuclear agreement actually has been built yet, the accord most definitely is being carried out, as the head of Nuclebras, Nogueira Batista, said.

More than 100 Brazilian scientists are taking specialised training in Germany, and the Brazilian government has allocated generous amounts of

funds to local universities to expand programmes in nuclear-related fields of science. German atomic technicians already are working in Brazil. Adverts in newspapers are seeking secretaries fluent in Portuguese and German. Six subsidiary firms have been set up under Nuclebras to implement the agreement. These companies are to operate in areas such as nuclear technology and engineering, heavy equipment manufacturing, uranium prospecting and mining, and isotope enrichment.

Financing arrangements already have been completed for the first two 1.3

million-kilowatt German reactors that will be built in Brazil, with knowhow and components to be supplied by Germany's Kraftwerke Union. Related contracts have been signed between other German and Brazilian companies and consortiums, private as well as governmental, for site clearing and preliminary building construction.

Unofficial calculations show that over \$1,000 million-worth of cruzeiros, marks and dollars have been spent or committed under the German-Brazilian nuclear agreement so far. □

USA

Talk about the weather

Unusual weather encourages discussion of weather modification. Colin Norman reports on the debate in the western United States

THE strange weather patterns which have brought freezing temperatures and record snowfalls to the eastern United States this winter have brought the opposite problem to many western states. A severe drought is threatening agricultural production in California and it may disrupt hydroelectric power supplies in the Pacific northwest. And in the Rocky Mountain states the snow cover is so thin that the spring runoff is unlikely to be sufficient for crop irrigation, ranching, industry and domestic needs. In short, while the east is bracing for possible floods when the thaw comes, the west is facing a debilitating drought.

One consequence of this topsy-turvy pattern is that considerable public interest has been rekindled in the use of weather modification techniques to try to increase snowfall on the western mountains before spring arrives. The most dramatic expression of such interest came in Colorado earlier this month, when the state legislature approved a bill to provide up to \$250,000 for cloud seeding operations in the state. A major effort is also under way in Utah, some seedings have taken place in California, Oregon and Washington state, and an extensive long-term weather modification programme is also under discussion for the upper Colorado basin. One product so far is the generation of a good deal of local controversy.

This year's anomalous weather patterns have been caused by a stationary ridge of high pressure off the west coast, which has disrupted the normal westerly winds. Usually, the prevailing westerlies bring winter storms in from

the Pacific to dump snow on the mountains in the western states, but this year the winds have been deflected northward and have looped through Canada to descend on the eastern United States. The consequences in the east have been dramatic; in the west they have been more subtle.

Very little rain or snow has fallen in the Pacific northwest since September, and some industries which depend on hydroelectricity may face power reductions of 25% by the end of this month. Fire hazards abound in forest areas which are usually under several feet of snow at this time of the year. In California, domestic water supplies are already being rationed in Marin County near San Francisco, and last week the Bureau of Reclamation (an agency in the Department of Interior) notified farmers in the San Joaquin Valley that their irrigation water may be cut by 75% later this year. California produces about 40% of the fresh fruits and vegetables consumed in the United States. In Utah and Colorado, snowfall has been about 30-40% of normal, and as a result ranchers are selling off cattle because they cannot get sufficient feed and the winter wheat crop may be in jeopardy.

Another, more distant problem is likely to arise because spring runoff from the Colorado Rockies supplies most of the water in the Colorado River, which in turn provides much of the irrigation and drinking water for parts of Arizona, California, Nevada and northwestern Mexico. Though water now in storage in Lake Powell behind the Glen Canyon Dam and in Lake Mead behind the Hoover Dam will be sufficient to meet downstream needs for at least a couple of years, if this year's weather patterns are repeated next winter, some serious problems could occur. Small wonder, therefore, that weather modification techniques are attracting attention.

They will not provide salvation, however.

The legislation in Colorado will provide funds for massive cloud seeding operations during the rest of this winter on the western slopes of the Rocky Mountains, using silver iodide generators on the ground. Some airborne seeding will also take place in the eastern plains. In Utah, routine cloud seeding, sponsored by the Utah Water Conservation Board, has been undertaken for the past three years, and this year it is being expanded to cover nearly two-thirds of the state.

It should be noted, however, that cloud seeding can only increase natural precipitation; it cannot create snow when the conditions are not right for snowfall. Thus, the Colorado and Utah cloud seeding efforts will only be conducted if westerly winds return to bring snow clouds to the Rockies. According to a spokesman for the Colorado Water Conservation Board, the seeding programme will, at best, increase spring runoff this year by about 200,000 acre feet, an amount which will certainly not wipe out the anticipated shortages.

In a normal year, spring runoff from the Colorado Rockies into the Colorado River amounts to about 11 or 12 million acre feet. This year, Colorado officials are estimating that the runoff will be down to about 7 million acre feet.

Although any contribution from cloud seeding would be a help, environmentalists in Colorado vigorously opposed the emergency legislation when it was under consideration in the state legislature. Kevin Markey, a representative of Friends of the Earth, said last week, for example, that "it isn't emergency cloud seeding legislation, it's emergency political legislation to let everyone know that the governor and legislature are doing something". Markey argues that the time and effort spent on cloud seeding would be better devoted to water conservation programmes.

Environmentalists are particularly concerned about plans for a possible

massive cloud seeding effort now under discussion in Colorado and Utah, which would entail regular seeding of winter storm clouds to try to increase spring runoff to the Colorado River each year and boost water supplies to the arid southwest. A pilot-scale seeding effort was conducted by the Bureau of Reclamation in southwestern Colorado between 1970 and 1975 to measure the impact of cloud seeding on snowfall, and to evaluate potential long-term environmental consequences of a major effort covering most of the state. The results of that pilot effort were released last month, and they are adding to the debate.

The pilot project came up with two seemingly contradictory conclusions. First, it indicated that regular seeding may increase the snowpack by about 10%. And second, it seemed to show

that there was no statistical difference in the amount of snowfall on days when seeding took place compared with days of natural snowfall. Officials in the Bureau of Reclamation explain the contradiction by arguing that the seeding sometimes increased snowfall outside the target area, and residual silver iodide in the air often increased snowfall on days after seeding took place. Some seeding also took place on days when the conditions were not right for snowfall, which masked the overall effect, they claim. The ambiguous results are adding fuel to the debate about the advisability of sinking large sums into seeding this year, however.

The pilot project ended in May 1975, and discussions are now under way between officials in Utah and Colorado and representatives of the Bureau of Reclamation over the possibility of

expanding the effort into a large-scale demonstration programme. The Bureau has no money in its budget to begin such a project, however, and the earliest it could be started would be the winter of 1978-79. In the meantime environmentalists, who fear possible long-term effects on wildlife, increased damage from avalanches and potential effects on rainfall downwind from the target area, will continue to urge that water conservation be supported instead.

Whatever the outcome of that debate, it will not help alleviate the anticipated drought this spring. That would require at least twice the average snowfall between now and April, a prospect which weather forecasters deem extremely unlikely. As Colorado's Governor, Richard Lam, stated when he signed the emergency cloud seeding legislation, "It's a roll of the dice". □

BRITAIN

Solar commitment

Britain moved another step in its efforts to implement a broad national energy policy with the announcement last week of a programme supporting the exploitation of solar power. Chris Sherwell reports

A POSITIVE but measured commitment to solar energy from the UK government; a positive but measured response from the country's solar energy lobby. That was the state of play at the end of last week after the Department of Energy (DEN) had unveiled its latest research and development programme, this time in the field of solar energy.

The announcement, neatly preceded a day earlier by a DEN junior minister's well-publicised visit to a solar heated house to reaffirm the commitment to explore alternative energies, was timed to coincide with the long-delayed publication of a report prepared for the DEN by its Energy Technology Support Unit (ETSU) based at Harwell*. In following earlier announcements in support of wave and geothermal power it marks another step towards comprehensive exploitation of renewable sources of energy. Reports on combined heat and power and on tidal energy are expected in the next couple of months.

The new commitment of resources to solar energy amounts to £3.6 million over four years, and is expected to bring total government expenditure in the area to about £6 million over the same period. The £3.6 million will be

concentrated on three principal activities: the use of solar energy for water and space heating (the ETSU report sees this as offering the best return), the collection of additional data on solar energy, and the biological conversion of solar energy.

DEN officials were reluctant to indicate precisely how it would be allocated. An important fraction will go to work on biological conversion, but the bulk of the money will be used to accelerate the development of suitable solar power appliances. The DEN's idea is to negotiate contracts with companies with satisfactory proposals, offering them up to 50% funding to reduce the risk involved and encourage the work.

Much of the support will also complement work done by the Department of the Environment, notably through the Building Research Establishment. DEN funds will not be used to finance R&D on photovoltaic devices, however. This is already being done under the aegis of the Department of Industry.

The International Solar Energy Society in Britain (UK-ISES), though welcoming the latest development as a "good starting point" and though in agreement with the balance struck by the DEN, points out that the UK commitment compares unfavourably both in amount and in time-scale with that in other countries.

The UK-ISES also points out that in its report released last year, *Solar Energy: a UK assessment*, it estimated that solar energy could make an energy contribution of 35 million tonnes coal equivalent (mtce) by the year 2020—about 10% of total energy needs. It

urged an immediate incremental budget devoted to solar energy, beginning at £2 million a year in 1976 and reaching £10 million by 1980.

Dr Walter Marshall, the DEN's Chief Scientific Adviser, citing the ETSU report, said solar energy could be making a contribution of "around 2% of our present requirements" within 25 years (some 7 mtce), an amount which could represent a "take-off point" and might be increased by a factor of about 10 over the ensuing 25 years or so. But it was hard to assess the real potential, he said, since the trends in fuel prices weren't known.

Dr Marshall paid tribute to the UK-ISES for the quality of its report. And in response to a question about the delay in the government's announcement of a programme and in publication of the ETSU report—research for it was completed 20 months ago, in July 1975—he acknowledged that he had made a mistake in not setting the wheels turning in the DEN in anticipation of a favourable verdict.

That seemed like a surprising admission. But in a country blessed with a surfeit of energy for the medium term from its oil, gas and coal resources and its existing nuclear programme, the error of judgment seems more understandable. Still, the UK-ISES emphasises the opportunities even to an energy exporter offered by solar energy investment—especially in the field of export.

The UK ISES's enthusiasm for solar energy is not based just on faith. In January alone it received five telephone calls a day and 253 written enquiries from non-members. The newly-won enthusiasm of the DEN cannot be so complete. There the real fight is between coal and nuclear power. □

*Solar energy: its potential contribution within the United Kingdom (London, HMSO, £3.00).

IN BRIEF

EEC safeguards progress

The complicated checking agreement between Euratom and the International Atomic Energy Agency (IAEA), reached some time ago, is now in force. With the exception of France, EEC members are now coordinating the application of IAEA and Euratom safeguards to their nuclear plants, and routine IAEA inspections will take place at the same time as Euratom inspections. There is a possibility that France will enter into its own trilateral agreement with the IAEA and Euratom. The progress on safeguards, announced last week, is expected to reduce the threat to the EEC's supplies of enriched uranium from North America. Officials in Toronto were reported last week as saying that Canada may soon resume uranium shipments to Europe.

Spacelab choice made

The European Space Agency (ESA) and NASA have chosen the 77 scientific and technological experiments to be carried on the first Spacelab mission which is scheduled for the second half of 1980. Available resources will be shared equally by NASA and ESA—61 of the experiments are European, 15 are American and one is Japanese. The flight will test both the performance of the system itself and the capability for space research. Prime targets for research will be the stratosphere and upper atmosphere, but there will also be experiments on materials processing, plasma physics, biology, astronomy and thermodynamics and lubrication. NASA and ESA next have to choose two payload specialists to work in the pressurised module; they should be selected in mid-1978.

Low-cost technology prize

The Swedish Inventors' Association is living up to its name with the announcement that it is inaugurating a highly original prize of at least \$55,000 for innovations which will help to solve sociological and economic problems in the third world.

The prize will be given for their prospective, not retrospective, benefits; as it will be awarded on the hundredth anniversary of the Association's foundation, in 1986, the competition will last almost ten years. The inventors want to concentrate the competitors' efforts on certain subject-areas. The first one, announced in Stockholm recently was chosen with the firewood shortage in mind and concerns innovations related to reafforestation and the rapid production and decentralised use of wood.

A book called *All Creatures Great and Small* has been on the best-seller lists for quite a while. I suppose that not all of its readers know that the verses from which the title is taken include the couplet: "The rich man in his castle, the poor man at his gate, God made them high or lowly, and ordered their estate." These lines are not best-sellers. The world is now a neighbourhood, and its inhabitants wish to shop at the same grocery as their customers whose material wants they supply. For, if manufactured products move all over the world, why should the food supply not be distributed more evenly? My tape-recorder was made in Singapore, its plug-in microphone in Japan, the cassettes in Mexico and Haiti. We grant that the race is to the swift, and the spoils to the victors, who do their best to hang on to the said spoils. However, it seems paradoxical that many people in the 'high income countries' are frantically trying to eat less, so as to live longer, and other people in impoverished lands are desperately trying to get enough food to stay alive.

Take a look at the harm done by over-eating, and at the problem of food shortages. In both cases, the central issue is calories. Too many calories lead to obesity whether they come from starch, sugar, fat or protein. Usually, however, it is carbohydrates and fats that produce obesity, with its frequent dividends of heart disease, adult diabetes and arteriosclerosis. There is a growing suspicion that risk of breast cancer and cancer of the large bowel may also be increased by high-fat diets. Obesity is not confined to inhabitants of de-

veloped countries; overeating is often one of the first responses made by people to increased availability of food in any country.

Students of diabetes have recorded that its incidence is low in populations consuming low-calorie diets, whether these are high in fat (Eskimos), high

Calorie-deficient diets in impoverished countries are characteristically low in protein, because the cheapest sources of calories are low in protein and high in starch. Cassava meal, with a dismal 1-2% of protein, is at the bottom of the heap. It is a mainstay of many African diets. The proteins in such high-starch foods are often deficient in essential amino acids. Plants are not in business to provide animals with the good life.

The 'big three' cereals, rice, wheat, and maize, will continue to dominate the world food supply. They are deficient in lysine, tryptophan and isoleucine. Rice and wheat lack vitamin A, but a million recommended daily allowances of synthetic vitamin A cost only \$250. The Economic Research Service of the US Department of Agriculture, counting calories, says, "about 0.15 kilograms daily of wheat, rice, corn, sorghum, or millet would provide 500 calories. If the estimated 460 million malnourished people in the world were each provided daily with additional grain equal to 500 calories, much of the world's malnutrition would be alleviated." Where is the grain to come from? Critics of the new, high-producing strains of wheat and rice have claimed that social upheavals resulting from disruption of traditional agriculture have sometimes followed the introduction of these strains. These upheavals are accompanied by requirements for more irrigation, chemical fertilisers, and pesticides. However, for a hungry person, there is no substitute for food and, as is painfully evident in California in 1977, for a thirsty plant there is no substitute for water.

Counting calories**THOMAS JUKES**

in protein (Masai) or high in carbohydrates (Indians, Zulus). Indians who move to Africa, and who then increase their caloric consumption, show a marked increase in diabetes. No longer can sleek-headed men of fat sleep soundly at night.

The diseases resulting from deprivation of food are grimmer than their counterparts in the overfed category.

correspondence

The key to the universe

SIR,—It is always dangerous to assume that others are less intelligent than oneself. In your editorial *That Was the Weak Force, That Was* (3 February) you concede that "the initiated" found a certain amount to admire in the recent two hour programme, *The Key to the Universe*, but you add, patronisingly, "the uninitiated, which must have been 99.9% of the audience, can only have been thoroughly confused . . .". You then stand cleverly on your head and accuse the production team of one fundamental mistake: "they underestimate the serious-mindedness of the audience".

The Key to the Universe, shown on BBC2 on Thursday, 27 January, had an audience of three million; Nigel Calders' book, written in association with the programme, was soon second in the *Sunday Times* best-seller list.

We believe the public has a right to know about all aspects of science, including the esoteric, and television has a duty to inform them. It is our experience that didactic programmes attract at most half-a-million viewers: far less if made in the patronising way you advocate. I assure you that we do not underestimate the serious-mindedness of our audience and we shall continue to cater for those whom you appear to dismiss as "the uninitiated".

PHILIP DALY

Head of Science and Features,
BBC Television

Lavoisier's originality

SIR,—We have as little reason to put Lavoisier's scientific reputation on trial as had the Revolutionary Tribunal to question his loyalty to France. Gonzalez (December 9, page 504) reports that Lavoisier probably received, in 1767, a copy of John Mayow's *Opera Omnia Medico-Physica* in a shipment of 118 books ordered for his personal library.

It is much too facile to assert that "Mayow recognised the existence of oxygen, which he called *Spiritus nitro aereo*, in 1674 . . ." One might as well say that Lucretius recognised the existence of atoms 1800 years before Dalton, or Caesalpinus the circulation of blood 50 years before Harvey. If Mayow's writings were known to the 24-year-old Lavoisier in 1767, they were also known to other scientists in the preceding 93 years, yet chemistry had to wait until the 1770s for the work of

Priestley, Scheele, and Lavoisier. The fact is that the idea of the *Spiritus nitro aereo* did not originate with Mayow, but derived, as Henry Guerlac has shown¹, from earlier observations concerning nitre (potassium nitrate).

As a component of gun powder, nitre was associated with explosive heat and light, but it was also associated with cold, which it produces as it is dissolved in water. An aerial spirit of nitre was therefore invoked to explain the atmospheric phenomena of thunder and lightning, explosions so often accompanied by cold rain or hail. Since ancient times it was known that air is required for the heat of fires (for example in the use of blow pipes), and for the maintenance of animal life. Aristotle and Galen both felt that air helped to cool (to moderate) the animal body's heat. Hence in the seventeenth century an aerial spirit of nitre could be thought to provide both the heat and the coolness that the body required.

At Oxford in the years immediately preceding the young Mayow's work, Boyle and Hooke established with their vacuum pump that both a burning flame and the animal body require air; Hooke showed that the function of breathing is to supply this air rather than to squeeze by its motions the blood through the lungs, and Lower demonstrated that the blood undergoes a colour change as it passes through the aerated lungs. Much of this Mayow records in his book. He also records his own important experiments demonstrating that the combustion of certain materials, as well as respiration under certain circumstances, is accompanied by a decrease in the volume of air in an enclosed vessel. The progress of the seventeenth century scientists in understanding respiration has been lucidly summarised by Leonard Wilson².

Mayow was close to a discovery of oxygen in thinking that only a special part of air is involved in combustion, and that only this part is contributed by air to the blood in respiration. But his nitro-aerial spirit does not qualify as oxygen for many reasons, the most important of which are:

- It is not an ordinary material of definite mass or density, for Mayow asserts that it continues to exist in a vacuum, where it accounts for the conduction of light. Mayow did not claim that he had ever isolated it or measured its amount, and he did not prescribe any methods for doing so.

- Its qualities are too much dependent upon the circumstances impressed upon it, as for example its state of motion, which is to say that, like so much of the chemistry prior to Lavoisier, the nature of the thing was determined by too many qualifying adjectives.

- It is invoked to explain too wide a variety of fundamental natural phenomena, for example the nature of the sun, the presence of intense cold in the middle reaches of the atmosphere, the blueness of the sky, the nature of fire, the elasticity of air, the colours of different solid materials, and so on³. Incidentally, Mayow knew nothing of red blood cells and their pigment, as Gonzalez seems to imply. His explanation for the redness imparted to blood by air was that nitro-aerial particles are often of that colour, as is shown by the red distillate (nitrogen dioxide) which can be liberated by the heating of acidic nitre.

Thus it is hardly fair to assert that Lavoisier's discovery of oxygen was anticipated by Mayow, or that Lavoisier was guilty of scientific cheating in failing to give credit to him. Lavoisier's conception of oxygen was very different from Mayow's idea of the nitro-aerial spirit. And, as Guerlac has pointed out⁴, Lavoisier's achievement was more fundamental even than the discovery of oxygen itself, for he was the first fully to realise that a host of material substances can exist in the gaseous state, and that the balance sheets of chemical processes must account for the materials of gases as well as of solids and liquids. Throughout his relatively short life, Lavoisier was recognised by his contemporaries as a man of exceptional genius. The day after Lavoisier was executed, the brilliant mathematician Lagrange remarked: "Only a moment to cut off that head, and a hundred years may not give us another like it".

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¹ Guerlac, H., "John Mayow and the aerial nitre," *Congrès International de l'Histoire des Sciences*, Actes VII, 332-349, (Paris, 1953). Also "The Poets' Nitre", *Isis* 45, 243-255 (1954).

² Wilson, L., "The Transformation of Ancient Concepts of Respiration in the Seventeenth Century," *Isis* 51, 161-172, (1960).

³ Mayow, J., *Medico-Physical Works* (Re-issue edition of The Alambic Club, Edinburgh, 1957, An English translation of *Opera Omnia Medico-Physica*, Oxford, 1674).

⁴ Guerlac, H., *Lavoisier: The Crucial Year* (Cornell University Press, Ithaca, 1961).

⁵ McKie, D., *Antoine Lavoisier*, 292 (Collier Books, New York, 1962. A reprint of the Schuman edition of 1952).

news and views

Membrane receptor–adenylate cyclase relationships

from Melvyn F. Greaves

It is now generally accepted that adenylate cyclase, through the production of cyclic AMP, plays a crucial part in the recognition and transduction of regulatory signals by cells. The biological responses this enzyme helps to elicit are legion: gene expression (in *E. coli*); social integration and differentiation (in slime moulds); glycogen production and fat lipolysis (in mammalian cells); catecholamine activity; and (perhaps) balding in up to half the human race. The diversity of these cellular responses raises the question of how cyclic AMP can satisfy the specificity demanded by recognition–response events.

Relating adenylate cyclase activity to specialised membrane recognition events and cellular responses may not pose too great a conceptual problem with respect to mature, or 'differentiated' cells. Cyclic AMP may simply tell a cell to go faster or slower, switch on or switch off, in response to a limited number of environmental signals complementary to a restricted number of cyclase-regulating receptors. In addition, the cell's response is itself restricted qualitatively. This view although simplistic is nicely supported by experiments with cholera toxin which show that stereotyped responses can be elicited by bypassing normal receptor-dependent recognition events¹. It is also well known that cyclic AMP itself (or its dibutyryl derivative) when added to cells can also elicit the same response as the cell's normal regulators.

More serious problems are posed by the possible role of adenylate cyclase in the induction of gene programming (that is, in early 'differentiation' events) and in individual cells equipped with multiple distinct cyclase-linked hormone receptors.

A comprehensive picture of adenylate cyclase function requires much more understanding than we now have of how this enzyme's activity is integrated with other membrane and cellular events (such as ion fluxes and compartmentalisation, changes in microfilaments, membrane fluidity, activity of guanyl cyclase and phos-

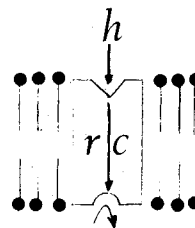
phodiesterases and regulatory processes of gene transcription and translation.

One key question is the nature of the relationship between the enzyme and receptor molecules themselves, a topic which has recently been the subject of much speculation^{2–7}. Figure 1 illustrates schematically the various

possibilities that have been considered. It is striking that much of the debate has been dominated by the presumption that physical coupling exists between receptor and cyclase.

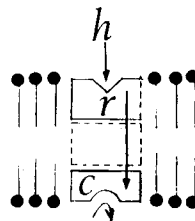
It is generally agreed that receptor and cyclase enzyme molecules reside mainly in opposite halves of the cell

A. Single molecule

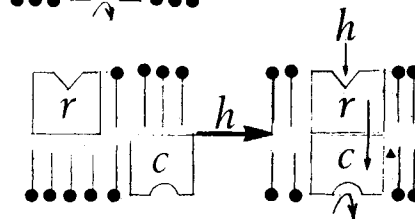


B. Physical coupling models

i. Stable association
tandem or triplet
schemes



ii. Motile receptor
hypothesis



C. Indirect interaction models

Key:
h: hormone
r: receptor
c: cyclase

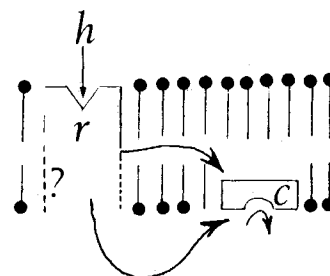


Fig. 1 Models of possible physical relationships between hormone receptors and adenylate cyclase.

surface membrane with their active or binding sites orientated towards the aqueous phases respectively outside and inside the cell⁸⁻¹². But the question of how they communicate remains. Are their 'ends' in contact with lipid as a lubricant or have they no physical continuity or proximity at all? What form does the modulation of cyclase activity take?

Following their extensive studies of adenylate cyclase regulation by the hormone glucagon and guanine nucleotides, Rodbell and colleagues^{12,13} have suggested some answers to the last question (see also a recent review by Helmreich *et al.*¹). They postulate that adenylate cyclase exists in three interconvertible states differing in catalytic activity and susceptibility to binding site inhibition by protonated substrate. Hormone-receptor interaction is thought to augment the normally slow (and spontaneous) transition of cyclase to that catalytic state in which it is least susceptible to inhibitory substrate. Binding of guanyl nucleotides to a regulatory site is thought to have a crucial intermediary or 'transducer' role in this hormone-facilitated transition. The favoured mechanism here, as in some other receptor systems, is allosteric interaction. Receptors are envisaged as regulatory molecules capable of exercising influence over alternative physical conformations of a partner 'effector' molecule (the cyclase enzyme or an intermediate). Hormone-receptor complexes then facilitate or stabilise that conformation of effector most commensurate with the appropriate biological response to the initiating ligand.

A 'mobile receptor' hypothesis for receptor-cyclase coupling has been proposed by Cuatrecasas^{4,14,15} who suggests that hormone-induced clustering of membrane receptors within the membrane facilitates contact and interaction with cyclase enzymes. This model derives its impetus from observations on lateral redistribution of exposed cell surface antigens (by antibodies), glycoproteins (by lectins) and gangliosides (by cholera toxin)¹⁶. A similar theoretical model has also been proposed recently by McGuire and Barber¹⁷.

The capacity of a particular receptor to be redistributed (by a hormone, for example) will depend, amongst other things, on receptor density, hormone concentration, the valency of interactions, the association of receptor with other molecules (such as lipids of varying degree of saturation and fluidity and submembrane microfilaments) and the overall organisational symmetry of the cell in terms of its orientation, association with neighbours and ordered non-random structure of its membrane. Binding studies with label-

led insulin suggest that receptors for this hormone may exist as small clusters¹⁴; however there is as yet no convincing demonstration of hormone-induced receptor clustering and there may be situations where the required redistribution is impossible.

Cuatrecasas¹⁴ cites the instance of morphologically-polarised epithelial cells (in gut, liver and bladder for example) in which hormone receptors and adenylate cyclase may normally be concentrated at opposite sides of the cell. He argues that this situation is quite incompatible with any form of permanent structural coupling and suggests that direct interaction between receptor and enzyme could be brought about by redistribution of the mobile hormone receptor. This mechanism requires the hormone-receptor complex to undertake a rather extensive journey around the sides of the cell towards the 'distal' pole (see Fig. 23 in ref. 14).

Although such large scale polarised redistribution is seen, and was of course discovered, in lymphocytes, these are motile cells with no intrinsic polarisation of receptors. Whatever mechanism of large scale redistribution is favoured^{16,19}, it seems rather unlikely that such membrane behaviour would be possible in non-motile tissue cells bound by various intercellular junctions. Indeed if the strict polarisation of receptors and adenylate cyclase is correct this situation provides a powerful argument against all models of receptor-cyclase interaction favouring physical coupling.

Alternative models which demand no physical coupling between receptor and cyclase enzyme must therefore be considered. Perkins², for example, has proposed that hormone receptors may modulate cyclase enzyme activity indirectly through a lipid 'field' effect of undefined nature.

An ingenious technique which may shed some light on receptor-cyclase relationships has been used recently by Orly and Schramm²⁰. These authors (using Sendai virus) fused turkey erythrocytes, in which the endogenous beta adrenergic receptor had been inactivated by heat or N-ethylmaleimide, with Friend erythroleukaemia cells. The latter have adenylate cyclase but no demonstrable beta adrenergic receptor. The objective of the fusion experiment was then to 'couple' the turkey erythrocytes' beta adrenergic receptor with the cyclase derived from the leukaemic cells. Within a few minutes of fusion adenylate cyclase activity (measured by cyclic AMP formation by cell ghosts) could indeed be considerably enhanced by isoproterenol. The levels of activity recorded with a single concentration of isoproterenol (50 μ M) were at least as high in the 'coupled hybrid' as in the untreated

turkey erythrocyte cells incubated with isoproterenol or sodium fluoride.

This system promises to be an exciting new way of investigating lesions in membrane receptor function. Does it tell us anything about the receptor-cyclase linkage itself? Orly and Schramm interpret their results in terms of the model developed principally by Rodbell and colleagues in which the hormone receptor interacts with cyclase by way of a guanyl nucleotide binding component. However, this still leaves open the question of the physical relationship of hormone binding site, guanyl nucleotide binding site and cyclase catalytic site. One broad conclusion which seems readily available from the fusion experiments is that whatever the physical receptor-cyclase relationship, it is established with remarkable speed and apparent ease. More specifically the experiment implies that the beta adrenergic receptor is not normally coupled to an endogenous cyclase which is sub-type specific or peculiar to this receptor. This would appear to accord with the earlier studies of Rodbell^{8,21} and others^{2,22} who demonstrated that several distinct hormone receptors appeared to 'compete' or have access to a common pool of adenylate cyclase. If correct these interpretations weaken any direct coupling models. Several other factors may also argue against a direct interaction of receptor and cyclase. Binding of some hormones (growth hormone for instance) to exceedingly few receptors, constituting a minute fraction of the cell surface area, can elicit within seconds dramatic effects of cell membrane activity such as conformational changes detected by spectroscopic methods and ion fluxes⁷.

Taking into consideration the efficacy of such small numbers of receptor molecules along with the ease and speed of functional receptor-cyclase recoupling in the hybrid experiments it seems very likely that some rapid amplification step occurs between hormone receptor molecules and adenylate cyclase modulation.

Sonnenberg and Schneider have recently proposed a comprehensive model for hormone action drawing heavily on analogy with neurotransmitters⁷. They suggest that multiple membrane-associated events involving enzyme activation and microfilament changes may follow, be dependent on, and be propagated by, rapid alterations in ion fluxes and electric potential initiated by a small number of receptors. For this model to be plausible there is no reason to suppose the cyclase enzyme has, or requires, physical contact with any part of the hormone receptor molecule. This model, like its predecessors, is a working hypothesis requiring verification or denial by experiment. As it

stands the idea has a seductive and perhaps illusory simplicity. If its basic thesis is correct then it may in fact be even more difficult to unravel the circuitry and causal relationships involved in membrane signal transduction processes than was earlier supposed. □

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Breakdown of superfluidity in liquid helium

from A. J. Leggett

It has been known for more than 60 years that liquid helium ('He) is superfluid below about 2 K; that is, it flows through narrow capillaries without observable friction, and, conversely, an object moving slowly through 'He at low temperatures experiences practically no friction either. A major step forward in understanding this phenomenon was made by the Russian physicist Lev Landau in 1941; he showed that superfluidity is a natural consequence of certain assumptions about the 'elementary excitations' of the liquid, and predicted the critical velocity above which normal behaviour should set in. In a recent paper (*Phil. Trans. R. Soc.* 284, 179; 1977) D. R. Allum *et al.* report the first unambiguous observation of this 'Landau critical velocity', thereby strongly confirming Landau's ideas. They also report some surprising and significant

features of the mechanism by which superfluidity breaks down above the critical velocity.

An 'elementary excitation' is the simplest (quantised) disturbance which can be created in a given system; generally, it will carry a momentum p and an energy E which depends on p . Landau argued that at sufficiently low temperatures a heavy external body travelling through the system can experience friction only if it can create elementary excitations. However, conservation of momentum and energy prevent this happening at velocities less than the Landau critical velocity v_L given by

$$v_L = \min(E(p)/p)$$

A slight modification of the argument gives the same critical velocity for helium flowing through a capillary. In the case of a normal classical liquid it is easy to show that v_L is zero, so there is no superfluidity.

For liquid helium below 2 K, however, it is believed that the elementary excitation spectrum (the graph of $E(p)$ against p) should have the form shown in the figure. The excitations of small p (long wavelength) are quantised sound waves, or phonons, whereas those near the minimum of the curve are called 'rotons'. Originally a roton was envisaged as a quantised rotational motion of the liquid, but more recent theories have suggested some modifications to this picture and its physical nature is still somewhat obscure. The existence of rotons and more generally the correctness of the $E(p)$ curve of the figure has been confirmed both by specific-heat data and by experiments in which a neutron is scattered from the liquid by creating a single elementary excitation.

It is obvious from the figure that the minimum of $E(p)/p$ occurs near the roton minimum in the curve and is in fact given by the slope of the straight line; numerically, this corresponds to a value of v_L of about 50 m s⁻¹. At velocities higher than this emission of one or more rotons will occur, giving rise to friction. However, experiments on the flow of helium through narrow capillaries usually yield much lower (and often geometry-dependent and/or irreproducible) critical velocities, probably owing to the existence of tangles of vorticity in the liquid.

Allum and coworkers used the alternative technique of measuring the frictional drag on bodies moving through the liquid—in this case, negative ions. (A negative ion in liquid helium is believed to be a complex object composed of an electron plus the cavity it excavates for itself in the liquid and the portion of the helium which moves with it; the whole has a diameter of the order of 10 Å and a

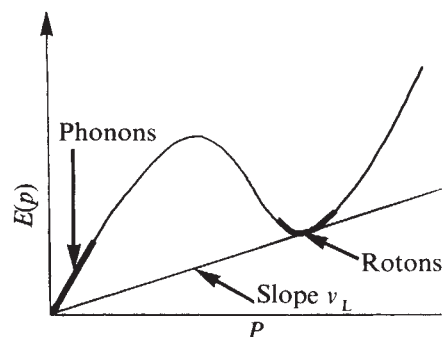


Fig. 1 Elementary excitation spectrum of superfluid helium.

mass of about 70 helium-atom masses). When plotted as a function of electric field E , the mean velocity of the ions, $\bar{v}$, rises virtually instantaneously from zero to a value which is of order 50 m s⁻¹ and coincides within the experimental error with the value of v_L predicted from the excitation spectrum. This indicates strongly that at velocities less than v_L there is indeed no frictional drag, in accordance with Landau's ideas.

A second interesting and quite unexpected result emerges from a comparison of the observed behaviour of $\bar{v}$ as a function of E with a recent theory, due to R. M. Bowley and F. W. Sheard, of the frictional drag at velocities above v_L . They showed that if the dominant process by which the ion loses energy and momentum is creation of single rotons, the quantity $\bar{v} - v_L$ should be proportional to $E^{2/3}$, whereas if it is creation of pairs of rotons which dominates it should be proportional to $E^{1/3}$. For most of the range of fields studied the data fit an $E^{1/3}$ dependent; they cannot possibly be reconciled with the $E^{2/3}$ one. Thus, it seems that negative ions moving in helium create rotons only, or at least predominantly, in pairs whereas neutrons seem to have no difficulty in creating them singly! This intriguing result seems certain to stimulate further research, both experimental and theoretical, into the physical nature of rotons. □

Dynamic Stark splitting

from Peter Knight

WORKERS in fields other than quantum optics or atomic physics may be surprised to hear that resonance fluorescence, that classic phenomenon of early quantum physics, has shrugged off its antiquarian associations to become a major and intensively studied new research problem. The rejuvenating influence has been the tunable laser. Unlike life, 'undressed' atoms, those old-fashioned atoms bathed in a weak

radiation field, become very much more interesting when 'dressed' by an intense resonant radiation field. The interest has shifted from a purely spectroscopic analysis of atomic structure to the basic mechanisms by which any atom interacts with radiation.

At low excitation intensities the process of resonance fluorescence is well understood: if the excitation field is monochromatic, the atom absorbs a photon at the excitation frequency, and energy conservation demands that the emitted photon have the same frequency. One-photon processes are dominant. The emitted fluorescence has the same spectral profile, and in particular the same narrow width, as the excitation. Resonance fluorescence with a spectral width narrower than the natural linewidth has been observed for the first time, by Gibbs and Venkatesan at Bell Laboratories (*Opt. Commun.* **17**, 87; 1976), using the dye-laser excited 2,852 Å ^{24}Mg transition, and by Wu, Grove and Ezekiel at the Massachusetts Institute of Technology (*Phys. Rev. Lett.* **35**, 1426; 1975), using the 5,890 Å D line of sodium.

Of more interest is what happens to the fluorescence process as the laser intensity increases. In a strong resonant field, non-linear scattering occurs. Multiphoton processes become as important as single photon processes. Energy conservation holds overall for the multiphoton process, but the fluorescence spectrum is no longer monochromatic. The atom can coherently interact many times with the laser field before spontaneously radiating a photon. Clearly we can no longer rely on the conventional low-order perturbation theory of resonance fluorescence.

An atom of principal transition frequency ω_0 interacting with a coherent laser field of frequency ω is stimulated to emit and reabsorb photons. The rate at which transitions are coherently induced between the two atomic levels is known as the Rabi frequency and is proportional to the square root of the laser intensity. One can think of the atom heuristically as a damped driven quantum oscillator, the damping being supplied by spontaneous emission and the driving force by the interaction with the laser field. At low intensities, spontaneous emission is more important than stimulated processes, and the atom behaves as an over-damped oscillator, radiating a field centred upon the driving frequency. At higher intensities the stimulated Rabi nutational frequency can exceed the spontaneous emission rate and the atom behaves just like an under-damped oscillator. The Rabi oscillations show up not only in the

population of the atomic levels (the inversion) but also as a modulation of the quantum dipole moment. This in turn leads to sidebands emerging in the spectrum of the emitted radiation, and this so-called "dynamic Stark splitting" has attracted a great deal of interest.

The basic theory to describe monochromatic strong-field resonance fluorescence was developed by Mollow some time ago (*Phys. Rev.* **188**, 1969; 1969). Mollow calculates the emission spectrum directly from the Fourier transform of the atomic dipole moment correlation function. This correlation function (if steady state is assumed) can be expressed in terms of the atomic inversion and dipole moment produced by the strong laser field interaction. Qualitatively we expect to find a strong-field fluorescence spectrum with a central spontaneous emission line, and two subsidiary sidebands separated from the central component by the Rabi frequency Ω , which is proportional to the amplitude of the laser electric field strength. Many authors have contributed to the detailed understanding of the fluorescence spectrum, but the broad features are all contained in the Mollow theory.

Experimental work by three groups all using dye-laser excited atomic beam methods broadly confirm Mollow's original prediction. Schuda, Stroud and Hercher (*J. Phys.* **B7**, L198; 1974), Ezekiel *et al.* (Wu, Grove & Ezekiel (*op. cit.*)), and Walther and coworkers at Köln and München (*Z. Physik.* **A278**, 205; 1976) have all observed the triplet splitting as the intensity increases. Certain asymmetries of the lineshape, almost certainly due to optical pumping effects extraneous to the basic phenomenon, have been noted and in part eliminated by careful preparation of the atoms by circularly polarised light.

Theoretical interest seems now to be concentrating on the role of the state of coherence of the incident laser radiation in these basically multiphoton processes. Intensity fluctuations will obviously be important as a 'smearing-out' effect: the a.c. Stark splitting is proportional to the square root of the instantaneous intensity. Similarly, phase fluctuations will create jitter in atomic dipole moments and correlation func-

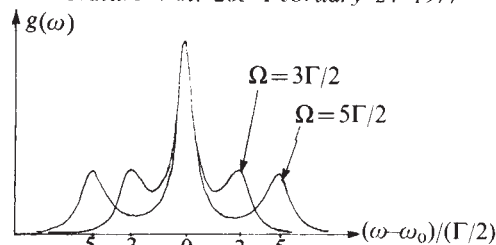


Fig. 1 The emission spectrum $g(\omega)$ for a resonantly driven two-level atom for two different laser intensities according to Mollow's theory.

tions will be additionally damped. To analyse the effects of a partially coherent field, and to generalise the purely monochromatic field approach of Mollow is not easy. The detailed dynamics of an atom interacting with an intense field are described by the optical Bloch equations (analogous to nuclear magnetic resonance equations of motion). These equations can be solved straightforwardly by a Laplace transform technique if the radiation field is purely monochromatic, but this is not directly applicable if the field is partially coherent. Neither do traditional Bloch-Wangsness relaxation methods apply, because the laser power is too high.

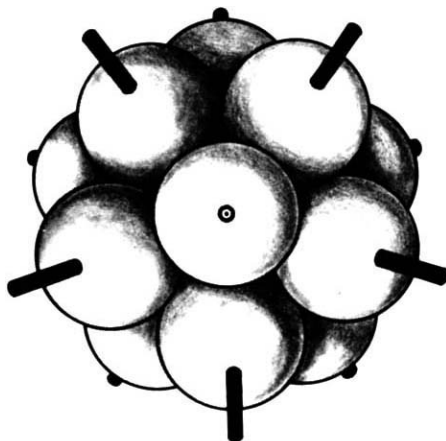
Recently, a non-perturbative theory of atomic relaxation in the presence of intense partially coherent radiation has been developed by Eberly (*Phys. Rev. Lett.* **37**, 1387; 1976) and applied to the theory of resonance fluorescence. By including stochastic phase and amplitude fluctuations of the laser directly in the equations of motion for the atomic dipole and inversion operators and assuming a simple exponential damping of first order phase and amplitude correlations, he describes how the Mollow theory of resonance fluorescence is modified by the finite bandwidth of the laser field. He is also able to include the effects of the interferometer analysing the radiated field spectrum. His results indicate that laser phase and amplitude fluctuations can alter the shape of the fluorescence spectrum in an unexpected and non-additive way. Amplitude fluctuations increase the height of the centre peak relative to the sidebands; phase fluctuations lower it. Eberly's results have been confirmed by Agarwal (*Phys. Rev. Lett.* **37**, 1383; 1976) using much more complex and physically less transparent methods. Agarwal goes on to look at the effect of laser bandwidth on the statistics of the emitted radiation. It is known from the work of Walls and others (Carmichael & Walls *J. Phys.* **B9**, 1199; 1976) that the emitted radiation should exhibit a fundamentally quantum feature called "antibunching", in contrast to the photon bunching familiar from the Hanbury-Brown Twiss experiment. Agarwal's results indicate that this too is affected by the laser bandwidth. □

A GREAT advance in DNA research is reported in this issue: the first complete nucleotide sequence of a DNA genome has been established by Sanger's group in Cambridge (see page 687 of this issue of *Nature*). For the first time it is possible to look at a viral genome as a giant molecule of known structure, all functions of which should be accessible to interpretation in terms of its molecular structure.

The small coliphage Φ X174 is particularly suitable for studies on the structural features underlying the complex processes of gene expression. It has a number of unusual characteristics—its small DNA possesses an unexpectedly high information content and seems to exploit every structural possibility to control gene expression. The single-stranded circular DNA genome of 5,375 nucleotides codes for nine proteins, some of which have been directly sequenced. The amino acid sequence of the others can be deduced from nucleotide sequence data. Genetic and physical maps of the genome have been constructed and have been correlated recently by Weisbeek *et al.* (*Virology* **72**, 61; 1976) who estimated the probable length of each gene. But there is a discrepancy between the calculated coding potential of some regions in the physical map and the proteins produced. Replication and transcription of the genome have also been studied extensively, with some unexpected results. Baas *et al.* (*J. molec. Biol.* **102**, 633; 1976) located the origin of replication in the middle of a structural gene coding for protein A. Hayashi *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **73**, 3519; 1976) reported the presence of two mRNAs covering the *A* gene region, only one of which was functionally active. The complete sequence has now provided a sound structural basis for these peculiarities.

Φ X174 DNA has been the subject of nucleotide sequence studies from early on. Practically all DNA sequencing techniques have been applied to this molecule, and some have been actually worked out on this DNA. Five years ago classical degradation techniques only allowed the determination of sequences of about 50 nucleotides which made the task of resolving the primary structure of DNA molecules seem much more difficult than the sequencing of RNA. Introduction of new techniques, making use of primed synthesis of highly-labelled complementary strands on Φ X DNA template greatly extended these possibilities. Eventually the new highly efficient 'plus and minus' technique of Sanger and Coulson (*J. molec. Biol.*

Φ X174 sequenced



from Maria Szekely

94, 441; 1975) which allows 150–200 nucleotide long sequences to be read directly, made it possible to produce a complete sequence of the Φ X genome a year after the first (and so far only) complete sequence of an RNA genome had been determined (Fiers *et al.* *Nature* **260**, 500; 1976).

Together with these sequence determinations, information on characteristic structural features of some functional sites of the DNA has also contributed to the construction of a 'chemical map' of the Φ X genome. 5'-terminal sequences of three mRNAs produced *in vitro* have been determined by Smith and Sinsheimer (*J. molec. Biol.* **103**, 699 and 711; 1976); ribosome binding sites on mRNAs corresponding to the initiation of genes *B*, *J*, *F* and *G*, have been sequenced by Ravetch *et al.* (page 698 of this issue). Homologies with other promoter and terminator sequences as well as the presence of purine tracts at all ribosome recognition sites helped to locate each gene in the sequence. At the same time, these homologies confirmed that the structure suggested for these control signals is generally valid.

The interesting question now arises of how the peculiar properties of Φ X can be interpreted in the knowledge of the primary structure. The most intriguing aspect is how the very high information content can be compressed into a small DNA molecule.

It now seems that this phage has several ways of ensuring the most economical use of its DNA. The most spectacular discovery has already been reported—the same stretch of DNA can code for two proteins which are translated in different reading frames

(*News and Views* **264**, 11; 1976). In 1976 Barrell *et al.* (*Nature* **264**, 34) found that the complete *E* gene was contained within the *D* gene region. A similar situation is reported now for genes *B* and *A*. Two papers in this issue provide evidence that gene *B* is contained within the region of gene *A*: Brown and Smith (page 695) locate the ribosome binding site for gene *B* within the gene *A* region, and Smith *et al.* (page 702) assign mutants in gene *A* and gene *B* to different reading frames in the overlapping area.

Another DNA stretch which fulfils multiple functions was found at the beginning of the *A* gene, where the 3'-terminal part of an mRNA which had started at an earlier promoter overlaps with the beginning of the mRNA for the *A* gene. The sequence of this DNA stretch suggests that a specific hairpin loop structure may be involved in the control mechanism which provides within this overlap a termination signal for one mRNA without interfering with the initiation of transcription of another mRNA. Readthrough at the termination signal may explain Hayashi's finding of two mRNAs covering this area. Two more overlaps occur at the initiation sites of proteins C and J, where the initiation codons overlap with the termination codons of the previous cistrons. The purine tracts preceding the initiation codon serve thus both as recognition sites for ribosomes and as coding sequences for another protein. Gene *A* seems the most versatile part of the genome: in addition to the multiple functions listed above, it also contains the origin of replication, and thus recognition sites for the proteins involved. The exact site of the origin has not been located, but the region in gene *A* which contains this site has been defined. Its sequence is less characteristic than could have been anticipated, it shows no symmetry or secondary structure, only a probable specific arrangement of an A–T rich stretch between the G–C rich stretches.

The multifunctional character of Φ X DNA may be a unique property displayed only by a few very small DNA viruses. Overlapping termination and initiation codons have also been detected, however, in the tryptophan messenger of *Escherichia coli* (Platt & Yanofski *Proc. natn. Acad. Sci. U.S.A.* **72**, 2399; 1975), the possibility thus exists that at least some of the above ways to compress more information into DNA may be of more general significance. If this were so, our ideas about the coding potential of DNA genomes would have to be fundamentally altered.

X-ray laser quest

from I. J. Spalding

FOR the alchemists it was the Philosopher's Stone, for the fusion specialist it's 'scientific break-even', but for some laser enthusiasts their chosen—and one hopes attainable—goal is the X-ray laser. The advent of high brightness, uniquely monochromatic, coherent light sources in the visible and infrared regions of the electromagnetic spectrum has already completely transformed many interferometric, scattering and spectroscopic techniques, and has opened up prospects for entirely novel applications. One might expect that the development of coherent X-ray sources would similarly revolutionise such tasks as high-resolution, time-resolved X-ray diagnostics and crystal structure determination.

Why is this goal difficult to achieve? One fundamental problem lies in the short life time of the upper levels of interest: the spontaneous life time for a 10 keV K-L transition in copper metal might typically be 10^{-15} s, implying the gargantuan pumping requirement of ~ 1 watt per atom! Several ingenious schemes have been proposed to provide such high pumping rates. These include Gudzenko and Shelepin's proposal for rapid recombination in a dense (laser-produced) plasma, Duguay and Rentzepis' photoionisation scheme, and McCorkle's suggestion for sweeping a high-current ion beam at the speed of light along an obliquely-oriented foil target.

Not surprisingly, such novel physical conditions may pose unusual theoretical problems. Bonifacio, Hopf, Meystre and Scully have recently reviewed quantum electrodynamic modifications to conventional small-signal gain theory which become necessary in a travelling wave X-ray laser where the pump behaves as a delta function swept at the velocity of light along the active medium. Depending on how fast the population inversion decays compared with the atomic polarisation, the amplification may be substantially smaller than that anticipated from semi-classical (Maxwell-Schrödinger) theory. Happily, large-signal pulse amplification becomes more favourable in the non-linear regime since coherence brightening, super radiant effects and power broadening (that is, broadening of energy levels due to the e.m. electric field) are predicted to reduce saturation effects.

Most X-ray laser experimenters have concentrated their attention on transitions in laser-generated plasmas at wavelengths in the extreme ultraviolet, where detailed numerical computations have shown that reasonable gains are

Transform faults

from Peter J. Smith

WHEN Wilson (*Nature* **207**, 343; 1965) originally defined transform faults, he suggested that the transform faults offsetting the oceanic ridges were directly inherited from the pattern of continental rifting which had led to the formation of the relevant plate boundary. But many Earth scientists have never been entirely happy with this view of the origin of ridge-to-ridge faulting. Nor have they been happy with the lumping together of all transform faults into a single class—a class which fails to distinguish between, for example, the long San Andreas fault and the shortest of faults offsetting the mid-Atlantic ridge.

A distinction is now made by Gilliland and Meyer (*Geol. Soc. Am. Bull.* **87**, 1127; 1976) who divide transform faults into 'boundary transform faults' and 'ridge transform faults'. Boundary transform faults are major faults genuinely inherited from the original rifting which gives rise to a plate and "are necessary initially to permit differential movement between discrete plates". They are thus contemporaneous with the formation of the plate. They are most likely to

be ridge-to-trench faults and least likely to be ridge-to-ridge, although the latter are not excluded. Examples of boundary transform faults quoted include the Alpine, Denali, New Guinea, Philippines and San Andreas faults and the Ninetyeast ridge.

By contrast, ridge transform faults occur only along oceanic ridges and are thus always ridge-to-ridge types. They are not inherited from initial rifting but (as wax models show) result from the spreading process; that is, they are contingent upon the existence of spreading. They are secondary features and thus not generally contemporaneous with plate formation. Indeed, in theory they are not necessary for plate boundary formation at all and do not always exist when spreading rates are very low.

A weak point in the argument seems to arise from the need to admit ridge-to-ridge faults into both major categories. For example, Gilliland and Meyer regard the San Andreas fault as a boundary transform fault, although they concede that "its real nature is not by any means certain". Apart from the size of this particular fault and the fact that it impinges on land, the precise grounds for distinguishing it from ridge-to-ridge faults in the ridge transform fault category are not made entirely clear.

achievable using the high power lasers now available. The plasma conditions and computer codes required are similar to those of interest in laser fusion. In 1974 Irons and Peacock reported population inversion between states of principal quantum number $n=2-5$ in an expanding low-density C^{+5} laser-produced plasma, and recently Dewhurst *et al.* (*Phys. Rev. Lett.* **37**, 1265; 1976) have demonstrated inversion of the $n=3$ to $n=2$ transition of CVI at 182 Å at densities which it is claimed are sufficiently high to permit (length) scaling to useful gains.

This approach relies on an extremely rapid expansion and cooling of a fully ionised plasma; recombination then leads to a preferential population of the upper states, and hence an inversion. In the work of Dewhurst *et al.* the target was a 5.3- μ m diameter carbon fibre supported vertically at the focus of a 0.15 J Nd-glass laser pulse of duration 1.4×10^{-10} s. The fibre was first shattered by a carefully chosen prepulse, and then irradiated by the main pulse having a contrast ratio exceeding 10^4 . The width of the main pulse was determined by the time needed to heat and ionise the dense cold plasma formed by the prepulse. The presence of inversion was deduced from the relative intensities of (time averaged) Lyman series lines, photo-

graphically recorded by an absolutely calibrated 2 m grazing incidence spectrograph. Space-averaged spectra could be obtained using a single laser shot, but an average of 30 exposures was required when the radial resolution was narrowed to 100 μ m. The radial variation of L_{α} , L_{β} , and L_{γ} , intensities accords well with their numerical model, and thus they compute a maximum gain-length product of 2×10^{-2} for their experiment. They estimate that a 100 J laser should permit a gain-length product of ~ 10 , and so permit laser operation in a travelling-wave mode, and that the gain should vary as $Z^{7.5}$, where Z is the charge number of the ion. However for $Z \geq 13$ laser compression would be needed to achieve the very high densities (and small radii) required.

Although these and other developments are encouraging it should be noted in conclusion that it seems unlikely that regenerative feedback in the extreme ultraviolet will be possible, and that the shortest wavelength coherent sources are currently produced by non-linear optical conversion in solids and gases. (The record is temporarily held by a group at the US Naval Research Laboratory, who have in this way generated 380 Å, the 28th harmonic of the Nd-YAG 1.06 μ m laser.) □

articles

Nucleotide sequence of bacteriophage Φ X174 DNA

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A DNA sequence for the genome of bacteriophage Φ X174 of approximately 5,375 nucleotides has been determined using the rapid and simple 'plus and minus' method. The sequence identifies many of the features responsible for the production of the proteins of the nine known genes of the organism, including initiation and termination sites for the proteins and RNAs. Two pairs of genes are coded by the same region of DNA using different reading frames.

THE genome of bacteriophage Φ X174 is a single-stranded, circular DNA of approximately 5,400 nucleotides coding for nine known proteins. The order of these genes, as determined by genetic techniques²⁻⁴, is *A-B-C-D-E-J-F-G-H*. Genes *F*, *G* and *H* code for structural proteins of the virus capsid, and gene *J* (as defined by sequence work) codes for a small basic protein that is also part of the virion. Gene *A* is required for double-stranded DNA replication and single-strand synthesis. Genes *B*, *C* and *D* are involved in the production of viral single-stranded DNA: however, the exact function of these gene products is not clear as they may either be involved directly in DNA synthesis or be required for DNA packaging, which is coupled with single-strand production. Gene *E* is responsible for lysis of the host.

The first nucleotide sequences established in Φ X were pyrimidine tracts⁵⁻⁷ obtained by the Burton and Petersen⁸ depurination procedure. The longer tracts could be obtained pure and sequences of up to 10 nucleotides were obtained. More recently Chadwell⁹ has improved the hydrazinolysis method to obtain the longer purine tracts. These results are included in the sequence given in Fig. 1.

More extensive Φ X sequences were obtained using partial degradation techniques, particularly with endonuclease IV (refs 10 and 11). Ziff *et al.*^{12,13} used this enzyme in conditions of partial hydrolysis to obtain fragments 50–200 nucleotides long which were purified as ³²P-labelled material by electrophoresis on polyacrylamide gels. The fragments came from the same region of the genome and the sequence of a 48-nucleotide long fragment (band 6, positions 1,047–1,094) was determined using mainly further degradation with endonuclease IV and partial exonuclease digestions.

Another 50-nucleotide long fragment was obtained by Robertson *et al.*¹⁴ as a ribosome binding site. The viral (or plus)

strand DNA of Φ X has the same sequence as the mRNA and, in certain conditions, will bind ribosomes so that a protected fragment can be isolated and sequenced. Only one major site was found. By comparison with the amino acid sequence data it was found that this ribosome binding site sequence coded for the initiation of the gene *G* protein¹⁵ (positions 2,362–2,413).

At this stage sequencing techniques using primed synthesis with DNA polymerase were being developed¹⁶ and Schott¹⁷ synthesised a decanucleotide with a sequence complementary to part of the ribosome binding site. This was used to prime into the intercistronic region between the *F* and *G* genes, using DNA polymerase and ³²P-labelled triphosphates¹⁸. The ribo-substitution technique¹⁶ facilitated the sequence determination of the labelled DNA produced. This decanucleotide-primed system was also used to develop the plus and minus method¹. Suitable synthetic primers are, however, difficult to prepare and as DNA fragments generated by restriction enzymes are more readily available these have been used for most of the work reported here.

Another approach to DNA sequencing is to make an RNA copy using RNA polymerase with α -³²P-labelled ribotriphosphates and then to determine the RNA sequence by more established methods. Blackburn^{19,20} used this approach on intact single-stranded Φ X and on fragments obtained by digestion with endonuclease IV or with restriction enzymes. Sedat *et al.*²¹ were extending their studies on the larger endonuclease IV fragments and their results, taken in conjunction with the transcription of the DNA fragments²⁰, amino acid sequence of the *F* protein²², and the plus and minus method results, made it possible to deduce a sequence of 281 nucleotides (positions 1,016–1,296, Fig. 1) within the *F* gene²³. Transcription of *Hind*II fragment 10, amino acid sequence data in the *G* gene, and the plus and minus method using *Hind*II fragments 2 and 10 as primers, gave a sequence of 195 nucleotides (positions 2,387–2,582, Fig. 1) at the N terminus of gene *G* (ref. 24).

The 'plus and minus' method

Further work on the Φ X sequence has been done using chiefly the plus and minus method primed with restriction fragments. Figure 2 shows the various restriction enzymes used and the fragment maps for each (refs 25–30 and C.A.H., submitted for publication, and N.L.B., C.A.H. and M.S., submitted for publication).

Figure 1 shows the combined results of the sequence work to date. The sequence is numbered from the single cleavage site of the restriction enzyme *Pst*I. As with other methods of sequencing nucleic acids, the plus and minus technique used by itself cannot be regarded as a completely reliable system and occasional errors may occur. Such errors and uncertainties can only

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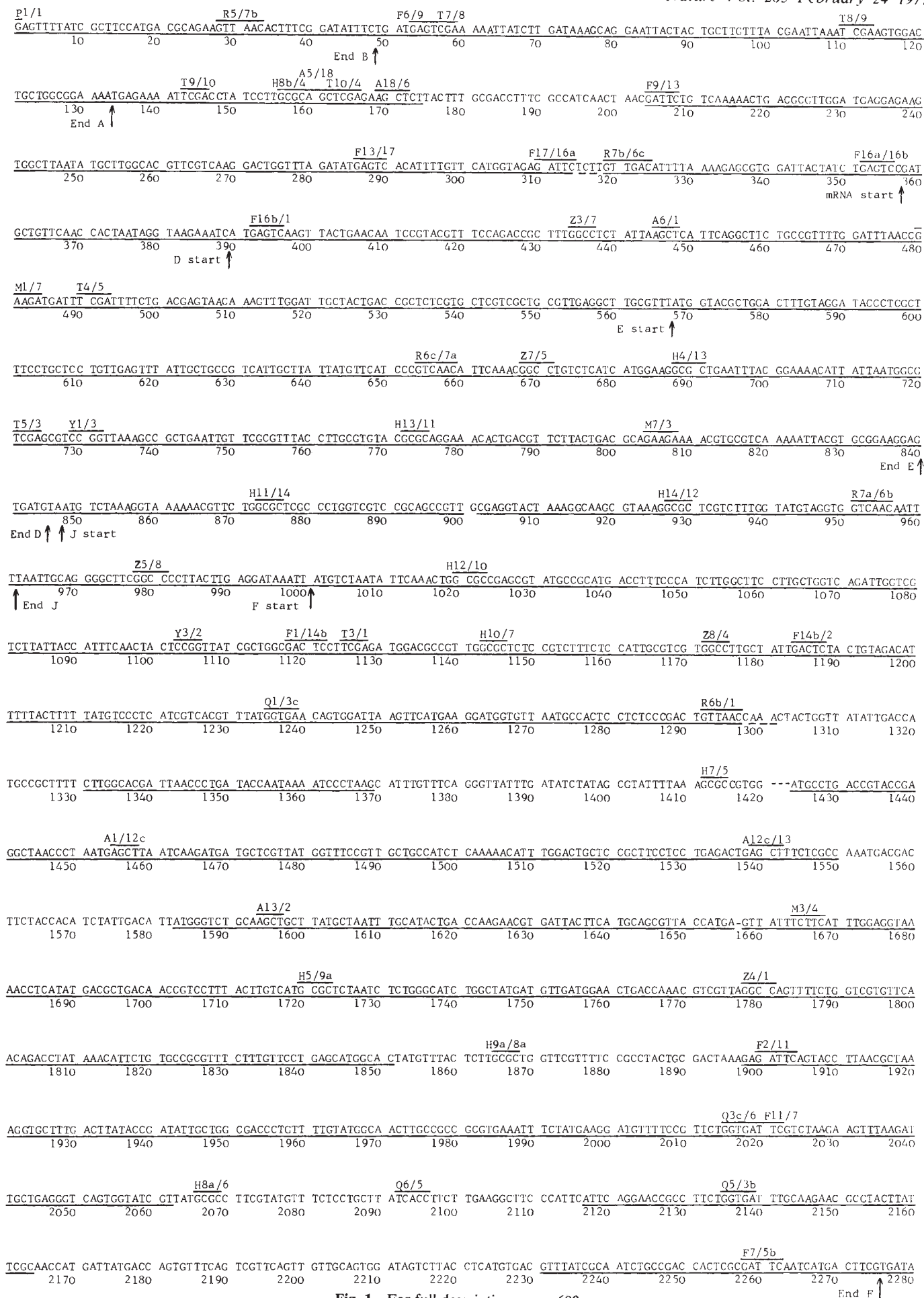
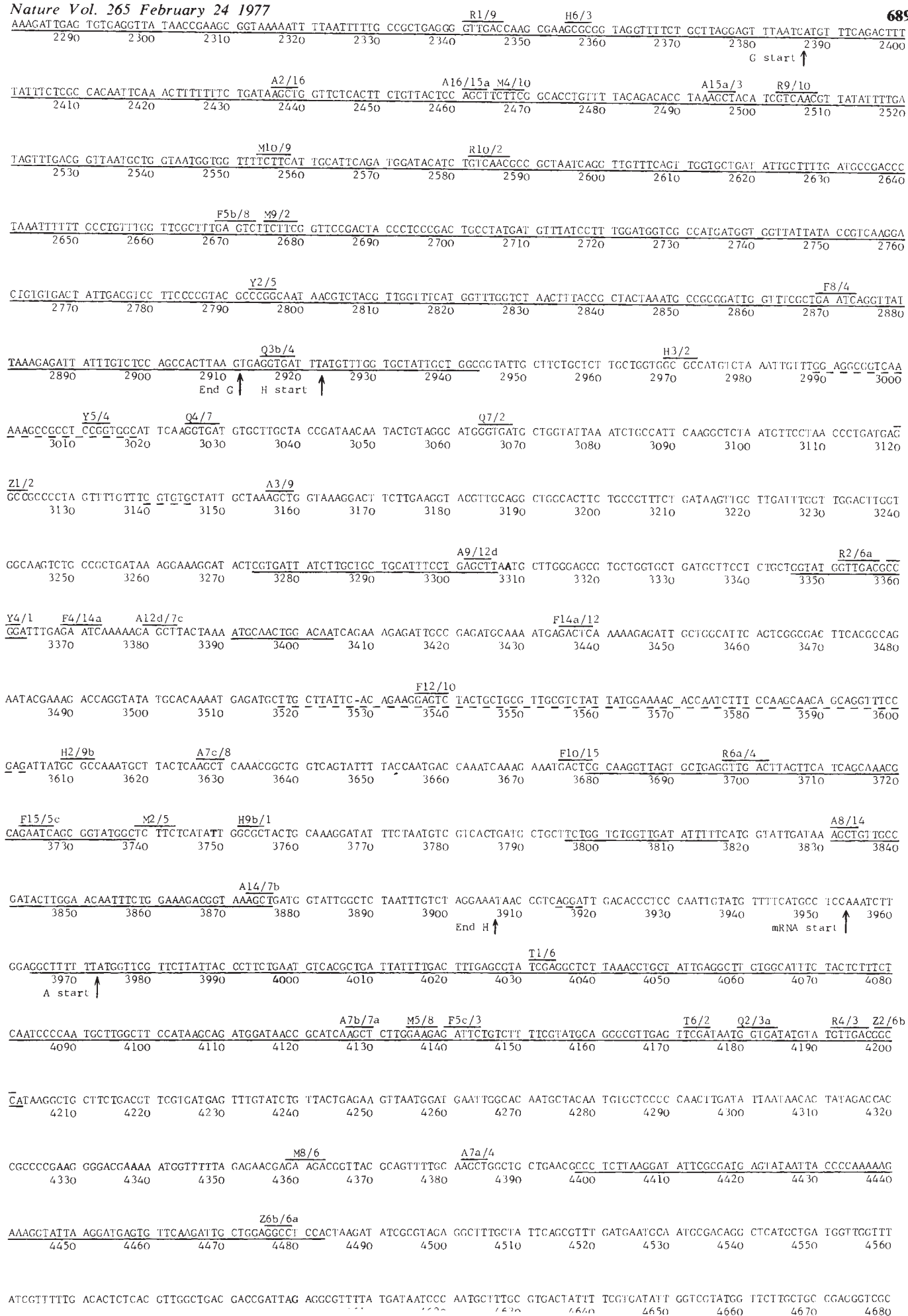


Fig. 1 For full description see p. 690.



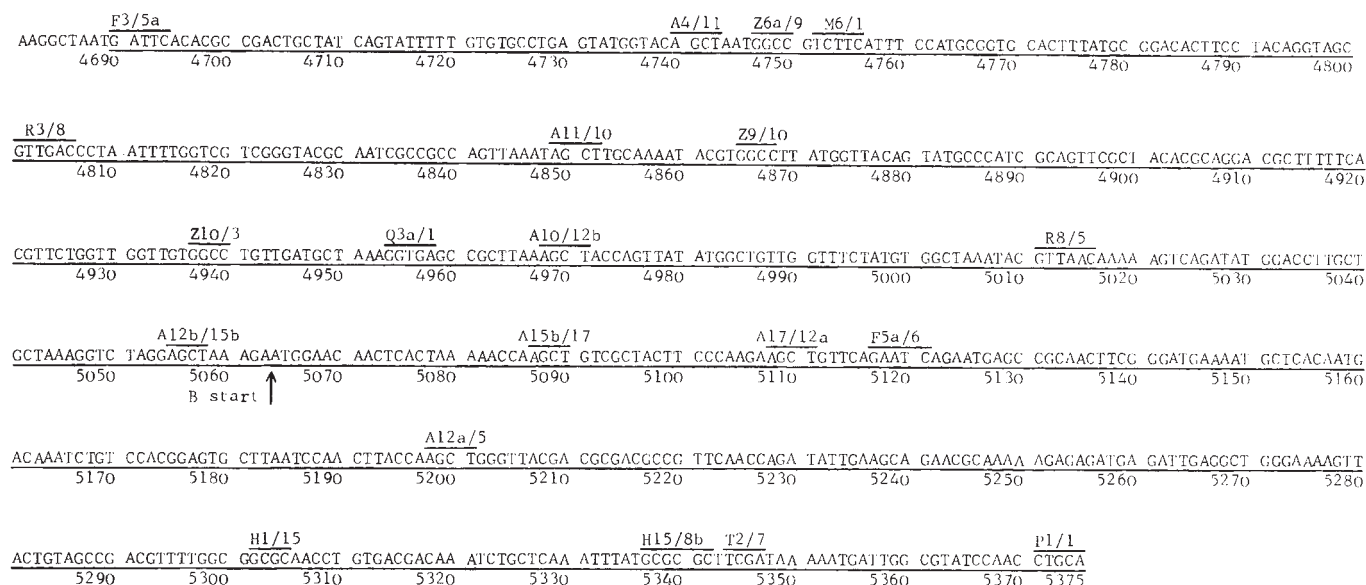


Fig. 1 A provisional nucleotide sequence for the DNA of bacteriophage Φ X174 *am3* *cs70*. Solid underlining indicates sequences that are fully confirmed; sequences with no underlining probably do not contain more than one mistake per 50 residues. Broken underlining indicates more uncertain sequences. Restriction enzyme recognition sites are indicated (for key to single letter enzyme code see legend to Fig. 2), as are mRNA starts and protein initiation and termination sites. Nucleotides 4,127 to 4,201 have been independently sequenced by van Mansfield *et al.*⁵⁸. The *am3* codon is at position 587.

be eliminated by more laborious experiments and, although much of the sequence has been so confirmed, it would probably be a long time before the complete sequence could be established. We are not certain that there is any scientific justification for establishing every detail and, as it is felt that the results may be useful to other workers, it has been decided to publish the sequence in its present form.

As template we have used both the viral (plus) and complementary (minus) strands of Φ X. Usually it is possible to determine a sequence with a single primer starting at about 15–100 nucleotides from the appropriate restriction enzyme site. In a particularly good experiment the sequence can be read out to 150–200 nucleotides but the results may become less reliable. Most sequences have been derived by priming on both strands; this allows more confidence than when only one strand could be used.

A useful method for confirming runs of the same nucleotide is depurination of 32 P-labelled small restriction enzyme fragments or of products of the DNA polymerase priming experiments (ref. 31 and N.L.B. and M.S., in preparation). The most satisfactory way of confirming the DNA sequences is through amino acid sequence data. As the methods used are entirely unrelated, the results of the two approaches complement each other very well and therefore complete sequences can usually be deduced from incomplete data obtained by each method. The complete sequence of genes *G* (ref. 32), *D* (ref. 33), *J* (ref. 33 and Freymeyer, unpublished) and most of *F* have been obtained in this way.

Many of the sequences in Fig. 1 have been amply confirmed and are regarded as established: these are indicated in the figure by underlining. Some sequences are considered to be reasonably accurate and probably contain no more than one mistake in every 50 nucleotides. Sequences that are particularly uncertain—either because of lack of data or conflicting results—are also indicated in Fig. 1.

In considering the sequence of Φ X174 as a functional unit it is convenient to begin in the region between the *H* and *A* genes and to continue around the DNA in the direction of transcription and translation.

A promoter and terminator

Sinsheimer *et al.*^{34,35} and Axelrod³⁶ have determined the sequences of the 5' end of three Φ X *in vitro* mRNA species and have located them on the restriction map. These sequences have been identified on the DNA sequence and one of them (AAATCTTGG) is found only at position 3,954 at which an *in vivo* unstable mRNA start has been located³⁷. The sequence to the left of this has some characteristics of typical *E. coli* promoters³⁸ in that five out of the 'ideal' TATPuATPu residues are present. Nearby, to the right of this mRNA initiation, however, is the sequence TTTTITA which is similar to sequences found at the 3' ends of a number of mRNAs (see ref. 39) and seems a likely signal for mRNA termination. The presence of a rho-independent termination site in this approximate position has been suggested^{36,37}, but the relative positions of the initiating and putative termination signals is rather surprising since the terminator for one mRNA would be expected to precede the initiator for the next. One possibility is that the T₆A might be acting as an 'attenuator' involved in the control of mRNA production in a similar manner to that suggested for the tryptophan operon by Bertrand *et al.*⁴⁰. If indeed it were acting as a transcription terminator one would expect a small RNA of 20 nucleotides to be produced, but no such product has yet been detected. Recent work, however, (Rosenberg, unpublished and ref. 41) indicates that termination may require the presence of a base-paired loop structure before the termination site. From the DNA sequence such a loop is probably present before the T₆A sequence, but in mRNA starting from the initiation site at position 3,954 this loop is not formed (Fig. 3). Therefore mRNA that had started at an earlier promoter and extended through the *H* gene would be expected to terminate here, whereas mRNA newly initiated at position 3,954 would not. This could be a way in which the phage has economised on the use of DNA—by having the ends of the two mRNAs overlapping.

The A protein

Where the amino acid sequence is available there is no problem in relating the DNA sequence to its coding properties, but it is

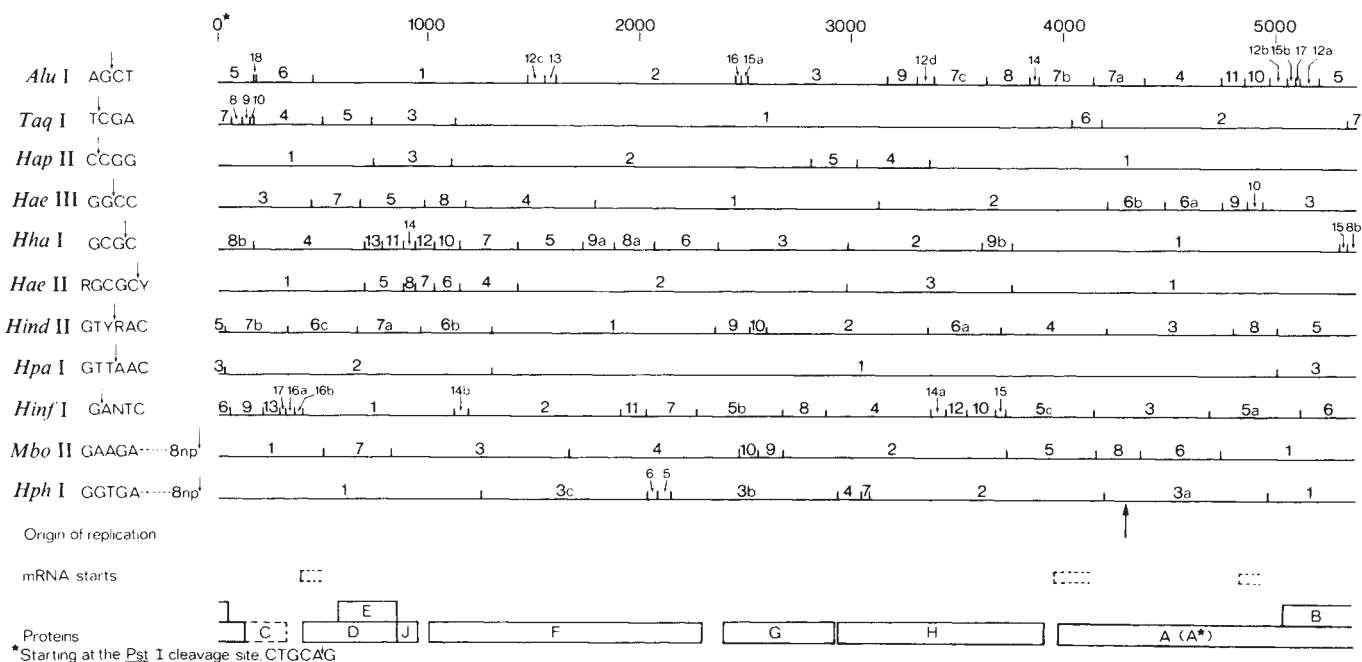


Fig. 2 Fragment maps of restriction enzymes used in the sequence analysis of Φ X174 am3 RFI DNA. Fragment maps of Φ X174 have been prepared for *Hind*II (R), *Hae*III (Z) and *Hpa*I+II by Lee and Sinsheimer²⁵, *Hin*HI and *Hap*II (Y) by Hayashi and Hayashi²⁶, and for *Alu*I (A) by Vereijken *et al.*²⁷ and for *Pst*I (P) by Brown and Smith³⁰. B.G.B., G.M.A., C.A.H. and D. Jaffe prepared the *Hinf*I (F) map, C.A.H. the *Hph*I (Q) map, and Jeppesen *et al.*²⁸ the *Hha*I (H), *Alu*I, *Hae*II and *Hap*II maps by using a rapid method depending on priming with DNA polymerase. A rapid two-dimensional hybridisation technique has been developed by C.A.H. (submitted for publication) and recently used for mapping *Mbo*II (M) (N.L.B., C.A.H., and M.S., submitted for publication) and *Taq*I (T)²⁹. *Hha*I and *Hinf*I maps have also been prepared by Baas *et al.*³².

more difficult to do so in the absence of such data, as is the case for the A protein. One way of identifying the reading phase of the DNA is from the distribution of nonsense codons. Over a sufficiently long sequence that is known to be coding for a single protein there is usually one phase that contains no nonsense codons, and this is identified as the reading phase. This requires completely accurate determination of the DNA sequences however: omission of a single nucleotide may give completely erroneous results. Another approach is possible in the case of Φ X. The results with the *F* and *G* genes^{23,24,32} showed an unexpectedly high frequency of codons ending in T. Therefore in a coding region there is a tendency for every third nucleotide to be a T and it is then possible to define the reading phase. Figure 4 illustrates how this characteristic was used to help determine the reading phase for the A protein and to identify its initiation codon at position 3,973. In a similar way the distribution of Ts may be used to identify errors in the DNA sequence, provided that such errors occur only infrequently.

A different approach to identifying the initiation site and reading phase in a coding sequence is by looking for a characteristic 'initiation sequence'. Shine and Dalgarno have shown that a common feature of ribosome binding sites is a number of nucleotides (at least three) preceding the ATG that are capable of forming base pairs with a sequence at the 3' end of 16S rRNA^{42,43}. All of the known initiation sites in Φ X174 that have been identified by direct amino acid sequencing (for the F, G, H, J and D proteins) satisfy this criterion (see Table 2) and the fact that the sequence preceding the ATG in position 3,973 also has this characteristic supports its identification as the initiation site for the A protein.

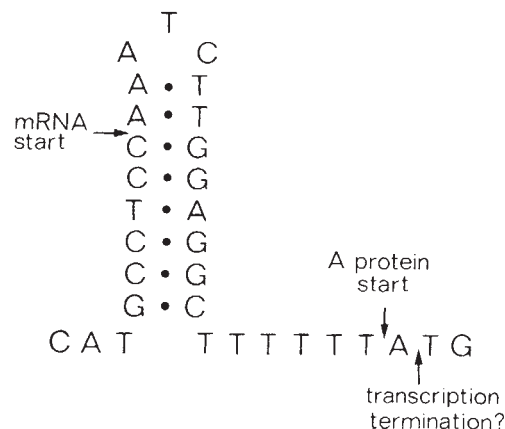
If, as has been suggested³⁷, some mRNA from the previous promoter does extend beyond the hairpin structure, initiation of A protein synthesis may be controlled by the inclusion of the region complementary to the 16S rRNA in the hairpin loop. This could explain the presence of two types of mRNA covering the A cistron, as suggested by Hayashi *et al.*³⁷—one unstable and active and the other stable but inactive. The former would be initiated at the A promoter, the latter at an earlier promoter and result from 'read-through' at the terminator. The postu-

lated reading frame for the A protein was confirmed by sequencing amber mutants mapping in the N-terminal region of gene A. *am86* proved to be a C→T change at position 4,108 and *am33* a C→T change at position 4,372. These both result in formation of an amber codon (TAG) in the same reading frame as the proposed initiating ATG and the sequence continues to the termination codon at position 133. The A protein, which is the largest coded by Φ X174, is thus 512 amino acids long with a molecular weight of 56,000, in good agreement with SDS gel estimations (see refs 4 and 44). The A* protein, with a molecular weight of about 35,000, is believed to result from an internal translational start in the A gene, in the same reading phase⁴⁵. From consideration of possible ribosome binding sequences^{42,43} the ATG in position 4,657 seems to be the most likely initiation site for the A* protein.

The origin of replication

The origin of Φ X viral strand DNA synthesis has been located in gene A, in restriction fragment Z6b (ref. 46). This origin, while

Fig. 3 Potential secondary structure at the A mRNA start.



	Ts in codon position		
	(1)	(2)	(3)
3,910 CCG.TCA.GGA.TTG.ACA.CCC.TCC.CAA.TTG.TAT.	5	2	1
3,940 GTT.TTC.ATG.CCT.CCA.AAT.CTT.GGA.GGC.TTT.	3	4	3*
3,970 TTT.ATG.GTT.CGT.TCT.TAT.TAC.CCT.TCT.GAA.	2	5	5
4,000 TGT.CAC.GCT.GAT.TAT.TTT.GAC.TTT.GAG.CGT.	3	5	7*
	5	3	7
	5	0	7*
	4	2	7

*These figures refer to the last five codons of the previous line and the first five of the next line.

Fig. 4 Identification of the initiation codon for the A protein. Sequences of 30 nucleotides in the region in which the initiation was expected were written down and arbitrarily marked off in triplets. The number of T residues in the first position in each triplet was then counted and listed, and similarly the number of Ts in the second and third positions. The marked preference for Ts in position 3 in the last two lines, as compared with the first two lines, suggests that they are coding for protein and that the triplets are correctly marked off. The most likely initiation codon for the A protein is the ATG in position 3,973.

coding for part of the A protein, probably corresponds to the position of the plus strand nick made by the same protein⁴⁴. Gaps in this region that are found in replicating double-stranded (RF) DNAs are probably related to the position of the nick. Eisenberg *et al.*⁴⁷ have investigated such gaps by depurination analysis and identified, in particular, the product C₆T. The sequence CTC₃ is found in position 4,285 (Fig. 1) and the location of the origin in this region agrees precisely with the results of Baas *et al.*⁴⁶. It is not possible at present to identify the actual position of the origin nick. The region shows no apparent secondary structure or symmetrical sequences, although there is an AT-rich region (4,298–4,307) between two GC-rich regions which might be of significance. Such a region is found near the origin of replication of SV40 DNA (ref. 48).

B promoter

The second of the mRNA 5' sequences (AUCGC)³⁴ has been mapped in restriction fragment R8 (Fig. 2), which starts about 300 nucleotides on from the proposed A* initiation. The sequence ATCGC is found at positions 4,832 and 4,888 in Fig. 1. The only way we can choose between them at the moment is that the second is preceded by the sequence TACAGTA (position 4,877), which is more akin to sequences found in known promoters³⁸ than are sequences preceding the other possible mRNA start. Irrespective of which of these sequences is used, the mRNA has a long 'leader' sequence (232

or 176 nucleotides) before the next proposed initiation codon (gene B).

The B protein

From a study of the ribonuclease T₁ digestion products of the ribosome binding sites of ΦX mRNAs⁴⁹, it was possible to identify an initiating ATG in position 5,064. From the genetic map^{2,3}, this would be expected to be gene B but, as discussed above, the A protein coding sequence extends right through this region, past the *Pst* site at residue 1 in Fig. 1, and terminates at residue 133. The initiating codon contained in the ribosome-protected sequence is, however, out of phase with the A protein reading frame. The proposed B protein coding sequence is one nucleotide to the left of the A protein phase, and continues until a termination codon occurs at position 49. Therefore the B protein coding sequence is totally contained within the A gene. These reading frames have been confirmed by sequencing mutants in genes A and B (*am16*, N.L.B. and M.S., in preparation; *am18*, *am35*, *ts116* (ref. 50)). Since the B protein has not been purified no protein sequence data is available. The complete amino acid sequence can be predicted from the DNA sequence however. The protein is 120 amino acids long with a molecular weight of 13,845 (including the N-terminal Met). The molecular weight estimates of the B protein obtained by SDS-gel electrophoresis are mostly greater than this (see review, ref. 4), but the electrophoretic mobility varied with gel concentration and cross linker. Such anomalous behaviour suggests that there may be, for instance, carbohydrate attached to the B protein.

The C protein

The next known gene product, protein C, maps between genes B and D. Examination of the DNA sequence in this region indicates that the most probable initiating ATG overlaps the termination codon, TGA, of gene A in the sequence ATGA at position 134. A possible termination codon for gene C could then be at position 391, although the sequence and phasing is not yet confirmed through this region. There is another possible protein initiation codon (position 51, overlapping the B protein termination codon) which would result in a slightly shorter gene product terminating at nucleotide 219. For the C protein, however, we favour the 'A terminator' start, since only this reading frame contains a CAA sequence, which by a C → T alteration could give the ochre 6 mutant. Ochre 6 is a gene C mutant produced by the decay of ³H-cytosine⁵¹ and has been mapped in fragments A6 and F9 (ref. 52); that is, between nucleotides 170 and 205 (Fig. 1).

Sequence following the D promoter

The mRNA 5' sequence which maps before the D gene (GAUGC)³⁴ is found at position 358 in Fig. 1. The sequence preceding the messenger start has only four of the TATPuATPu nucleotides³⁸. Thirty-two nucleotides after the mRNA initiation is the ATG (position 390) that initiates D protein synthesis. The amino acid sequence of the D protein has been determined

Table 1 ΦX174 coding capacity

Gene	Protein molecular weight from SDS gels*	Number of nucleotides (Fig. 1)	Protein molecular weight from sequence information
A	55,000–67,000	1,536	56,000
(A*)	35,000		
B	19,000–25,000	(360)†	13,845‡
C	7,000		
D	14,500	456	16,811‡
E	10,000–17,500	(273)†	9,940
J	5,000	114	4,097‡
F	48,000	1,275	46,400
G	19,000	525	19,053‡
H	37,000	984	35,800
Non-coding and C		485	
Total		5,375	

*See ref. 4.

†Values in parenthesis are overlapping sequences and therefore not included in the addition to obtain the total length of DNA.

‡These values are calculated from the amino acid sequence (in the case of B deduced from the nucleotide sequence). The others are derived using the formula

$$\text{Protein molecular weight} = \frac{\text{No. of nucleotides}}{3 \times 0.00915}$$

Table 2 Initiation sequences of Φ X174 coded proteins

D	C-C-A-C-T- <u>A-A-T</u> - <u>A-G-G-T</u> -A-A-G-A-A-T-C-A- <u>T-G</u> -A-G-T-C-A-A-G-T-T-A-C-T	Ser Gln Val Thr
E	C-T-G-C-G-T-T- <u>G-A-G-G</u> -C-T-T-G-C-G-T-T-T-A- <u>T-G</u> -G-T-A-C-G-C-T-G-G-A-C-T	
J	C-G-T-G-C-G-G- <u>A-A-G-G-A-G</u> - <u>T-G-A-T</u> -G-T-A- <u>T-G</u> -T-C-T-A-A-A-G-G-T-A-A-A	Ser Lys Gly Lys
F	C-C-C-T-T-A-C-T-T-G- <u>A-G-G-A</u> -T-A-A-A-T-T-A- <u>T-G</u> -T-C-T-A-A-T-A-T-T-C-A-A	Ser Asn Ile Gln
G	T-T-C-T-G-C-T-T- <u>A-G-G-A-G</u> -T-T-T-A-A-T-C-A- <u>T-G</u> -T-T-T-C-A-G-A-C-T-T-T-T	Met Phe Gln Thr Phe
H	C-C-A-C-T- <u>T-A-A-G</u> - <u>T-G-A-G-G-T-G-A-T</u> -T-T-A- <u>T-G</u> -T-T-T-G-G-T-G-C-T-A-T-T	Met Phe Gly Ala Ile
A	C-A-A-A-T-C-T-T- <u>G-G-A-G-G</u> -C-T-T-T-T-T-T-A- <u>T-G</u> -G-T-T-C-G-T-T-C-T-T-A-T	
B	A-A-A-G-G-T-C-T- <u>A-G-G-A-G</u> -C-T-A-A-A-G-A- <u>T-G</u> -G-A-A-C-A-A-C-T-C-A-C-T	
16S RNA 3' end	HO-A-U-U-C-C-U-C-C-A-C-U-A-G	

Where the protein start has been independently confirmed by protein sequencing data the amino acid sequences are indicated. The other initiation regions were identified as described in the text. Sequences complementary to the 3' end of 16S rRNA (refs 42, 43) are boxed; broken lines indicate further complementarity if some nucleotides are looped out or not matched. Ribosome binding to mRNA has been demonstrated in these regions for genes *J*, *F*, *G* and *B* (ref. 49).

almost completely, and nucleotide and amino acid sequences can be correlated to the termination codon at position 846 (ref. 33). The D protein, which is involved in capsid assembly, is 151 amino acids in length, with a molecular weight of 16,811. The D termination codon overlaps the initiation codon for gene *J* in the sequence TAATG. A similar structure has also been found by Platt and Yanofsky⁵³ in the tryptophan operon. The DNA sequence following this initiation codon matches the amino acid

sequence of the small basic protein (37 amino acids) of the virion determined by D. Freymeyer, P. R. Shank, T. Vanaman, C.A.H. and M. H. Edgell (personal communication). Benbow *et al.*^{2,3} suggested that the mutation *am6* was located in a gene *J*, coding for the small protein of the virion, and mapping immediately before gene *F*. Although marker rescue experiments indicate that *am6* is not in this region³⁴, the DNA sequence shows that there is a gene coding for the virion protein and we

Table 3 Promoter sequences in Φ X174

A promoter	A-G-G-A-T-T-G-A-C-A-C-C-T-C-C-C-A-A-T-T-G-T-A-T-G-T-T-T-T-C-A-T-G-T-T-T-T-C-A-T-G-T-C-T-C-C-A-A-A-T-C-T	3954 ↑ 18 nucleotides to A protein start
D promoter	<u>R7b/R6c</u> G-T-T-G-A-C-A-T-T-T-T-A-A-A-A-G-A-G-C-G-T-G-G-A-T-T-A-C-T-A-T-C-T-G-A-T-G-T-C-C-G-A-T-G-C-T	358 ↑ 32 nucleotides to D protein start
B promoter?	<u>R3/R8</u> C-A-G-G-T-A-G-C-G-T-T-G-A-C-C-C-T-A-A-T-T-T-T-G-G-T-C-G-T-T-C-G-G-G-T-A-T-G-C-A-A-T-C-G-C-C	4832 ↑ 232 nucleotides to B protein start
B promoter?	A-G-C-T-T-G-C-A-A-A-A-T-A-C-G-T-G-G-C-C-T-T-A-T-G-G-T-T-A-C-A-G-T-A-T-G-C-C-C-A-T-C-G-C-A	4888 ↑ 176 nucleotides to B protein start

mRNA initiation sequences³⁴⁻³⁶ are underlined. Boxed regions indicate sequences that may correspond to the TATPuATPu sequence found in other promoters³⁸, taking into account the distance from the mRNA starts.

have defined this as gene *J* (ref. 33). Since the *J* initiation codon overlaps the *D* termination codon we had to look elsewhere for gene *E*, which genetic mapping^{2,3} had placed between them. Amber mutants in gene *E* (*am3*, *am27*, *am34* and *amN11*) were located by the marker rescue technique and sequenced. All were found to be within the *D* coding sequence, with the mutant amber codons one nucleotide to the right of the *D* reading frame³³. Thus the *E* coding sequence is completely contained within the *D* coding region but in a different reading frame. The proposed initiation and termination codons for the *E* protein are at nucleotides 568 and 840, respectively³³, giving a protein 91 amino acids in length with a molecular weight of about 9,900 (including the N-terminal methionine).

The F protein

Following the *J* gene is an intercistronic region of 39 nucleotides before initiation of the F protein. There is no known function of this apparently untranslated sequence, although the presence of a hairpin structure (positions 969–984) suggests that it could be the site of the *in vivo* messenger termination signal³⁷ mapped in this region. The F protein is initiated by the ATG at position 1,001. This is the capsid component of the virion, and almost all the amino acid sequence is known^{22,24}. There are regions in this gene where the DNA sequence is not completely established, but the protein is about 424 amino acids in length, giving a molecular weight of $\approx 46,300$.

The G protein region

The termination signal for the F protein (position 2,276) is followed by an unusually long untranslated sequence of 111 nucleotides until the G protein initiation codon³¹. This region contains a looped structure which was postulated to have some functional role, as yet unknown, in the single-stranded DNA or the mRNA.

Initiation of the G protein at position 2,387 is followed by a sequence of 425 nucleotides until termination at position 2,912, giving a spike protein of molecular weight 19,053. The nucleotide and amino acid sequences of this gene and product are known^{24,32}.

The H protein

The initiation codon for the H protein (position 2,923) was identified first on the basis of the distribution of T nucleotides between the three reading phases, and later confirmed by amino acid sequence analysis. Amino acid sequence data on the H protein is minimal but the five peptide sequences known do correspond to the amino acid sequence, deduced from the DNA sequence by using the high frequency of third position T to help in assigning a reading frame to any given region. The DNA sequence is not entirely confirmed but it is possible to write a

reasonably accurate amino acid sequence for the H protein. The protein terminates at nucleotide 3,907, in agreement with carboxypeptidase results, giving a spike protein of molecular weight $\approx 35,600$ (326 amino acids). The amino acid sequence at the N terminus seems to be particularly rich in hydrophobic residues, which is consistent with its suggested function as the 'pilot' protein that reacts with the bacterial membrane^{55,56}. After H protein termination there are 66 nucleotides before initiation of the A protein at position 3,973.

Coding capacity of the ϕ X174 genome

The most striking feature of the ϕ X DNA sequence is the way in which the various functions of the genome are compressed within the 5,375 nucleotides. Since the identification of ϕ X gene products^{2,4} it has been clear that proteins of the accepted molecular weights could not be separately coded on the available length of DNA. However, with the presence of two pairs of overlapping genes (*B* within *A* (ref. 50), *E* within *D* (ref. 33)) the genome has more coding capacity than had been originally supposed on the assumption that each gene was physically separate. Table 1 summarises the molecular weights of the known ϕ X-coded proteins. There are other potential initiation sites for polypeptide synthesis (for example, in genes *A*, *F*, *G* and *H*) and further genetic work may clarify whether there are in fact other ϕ X genes as yet unidentified.

Initiation of protein synthesis

Table 2 lists the protein initiation sequences for genes *A*, *B*, *D*, *E*, *J*, *F*, *G* and *H*. It can be noted that there are no extra precursor sequences in proteins D, J, F, G or H at either the N or C terminus. There seems to be no relationship between the degree of complementarity to the 16S rRNA and the amount of protein synthesised, and we see no other features in the sequence that could explain different efficiencies of translation except where genes overlap.

Transcription of ϕ X174

The sequences preceding known mRNA starts^{34–36} are shown in Table 3. Other studies on promoter sequences³⁸ have suggested certain features that they may have in common. Although some of these features are present in the sequences preceding the ϕ X transcription initiations others are not, and at present it is difficult to suggest what signal on the DNA determines a promoter site or the efficiency with which it initiates RNA synthesis. It is interesting to note that a polymerase binding site found by Chen *et al.*⁵⁷, but not associated with any *in vitro* or *in vivo* mRNA starts, mapped near the region where there is the sequence TATGATG characteristic of promoters³⁸ (positions 2,705–2,711).

Table 4 Codons used in ϕ X174

Phe	TTT	39	Ser	TCT	35	Tyr	TAT	36	Cys	TGT	12
	TTC	26		TCC	9		TAC	15		TGC	10
Leu	TTA	19		TCA	16	Ter	TAA	3	Ter	TGA	5
	TTG	26		TCG	14		TAG	0	Trp	TGG	16
Leu	CTT	36	Pro	CCT	34	His	CAT	16	Arg	CGT	40
	CTC	15		CCC	6		CAC	7		CGC	29
	CTA	3		CCA	6	Gln	CAA	27		CGA	4
	CTG	24		CCG	21		CAG	34		CGG	8
Ile	ATT	45	Thr	ACT	40	Asn	AAT	37	Ser	AGT	9
	ATC	12		ACC	18		AAC	25		AGC	5
	ATA	2		ACA	13	Lys	AAA	47	Arg	AGA	6
Met	ATG	42		ACG	19		AAG	31		AGG	1
Val	GTT	53	Ala	GCT	64	Asp	GAT	44	Gly	GGT	38
	GTC	14		GCC	17		GAC	35		GGC	28
	GTA	10		GCA	12	Glu	GAA	27		GGA	13
	GTG	11		GCG	12		GAG	34		GGG	3

The totals are derived from sequences in Fig. 1 which are fully confirmed, that is, 377 codons in gene *A*, 120 in gene *B*, 152 in gene *D*, 91 in gene *E*, 38 in gene *J*, 344 in gene *F*, 175 in gene *G* and 49 in gene *H*. Out of a total of 1,346 codons 42.9% terminate in T. The percentages in the different genes are: *A*, 37.1 (non-overlapping region 47.1; overlapping region 15.8); *B*, 34.2; *D*, 42.1; *E*, 14.3; *J*, 47.4; *F*, 52.0; *G*, 54.3; *H*, 49.0. The initiating ATG is included in all cases.

The use of codons in Φ X174

Table 4 shows the codons used in regions where the nucleotide sequence is fully confirmed. It is clear that the pattern established by early observations on non-random use of codons^{23,24} is continued now that more information is available. In particular, the preference for T at the third position of the codon is marked throughout the genome, as shown in Table 4. In regions of overlapping genes, one of the pair tends to continue the 'third T' trend (*D* and *B*), thus excluding the other (*E* and *A*). This may give some indication of the order in which overlapping genes evolved^{33,50}. Another interesting feature is the very low occurrence of codons starting AG, particularly in non-overlapping regions. The base composition of the sequence of Φ X174 DNA shown in Fig. 1 is: A, 23.9%; C, 21.5%; G, 23.3% and T, 31.2%. This is in good agreement with previously determined values (see ref. 4).

We thank D. McCallum and R. Staden for carrying out the computer data storage and analysis of the sequence.

Note added in proof: J. E. Sims and D. Dressler (personal communication) have independently determined the sequence in positions 263–375 and 4,801–4,940. Their results agree with those given in Fig. 1. They have also identified the 'B' mRNA start as being at position 4,888.

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DNA sequence of a region of the Φ X174 genome coding for a ribosome binding site

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The DNA region corresponding to a newly identified ribosome binding site in Φ X174 DNA is sequenced and mapped in relation to the physical map of Φ X174. Assignments of the binding site to a specific gene are discussed, and the possibility of a second case of overlapping genes in Φ X174 is considered.

RECENT studies on the DNA of bacteriophage Φ X174 have defined sequences corresponding to the ribosome binding sites of genes *D*, *J*, *F* and *G* (refs 1–3 and B. G. Barrell, personal communication). A ribosome binding site that does not correspond to any of the above sequences has now been defined by studies on a ribosome-protected fragment of a transcript *in vitro* of Φ X174 RF DNA (ref. 4). Inspection of preliminary DNA sequence data obtained from Φ X174 (F. Sanger, *et al.*, unpublished) for potential ribosome binding sites, and comparison of the sequence data with T₁-oligonucleotides from the unassigned RNA fragment⁴, allowed identification of the

DNA sequence corresponding to this RNA fragment. We report here the complete DNA sequence of a region of Φ X174 *am3* RF DNA that corresponds to this new ribosome binding site. Because the DNA sequence contains accurately mapped cleavage sites for restriction endonucleases, the ribosome binding site can be located precisely on the physical map of the Φ X genome⁵, and therefore located on the genetic map^{6–8}.

Determination of the DNA sequence

The ribosome binding site lies in that portion of *Hind*II fragment 5 that overlaps *Hinf*I fragment 5a (nucleotides 5,000–5,100 approx, Fig. 1). The DNA sequence of this region was determined using the 'plus-minus' technique of Sanger and Coulson⁹ (Fig. 2). It was quantitatively confirmed by characterisation of the pyrimidine oligonucleotides obtained by primed synthesis *in vitro* of DNA labelled with α -³²P-dATP or α -³²P-dGTP using viral or complementary strand as template^{10,11} (Fig. 3, Table 1).

The sequence between the *Hind*II 8/5 cleavage site and the *Hinf*I 5a/6 cleavage site contains 111 nucleotides. It also contains the *Alu*I cleavage sites between fragments 12b, 15b, 17 and 12a (Fig. 1). The sequence determination allowed the

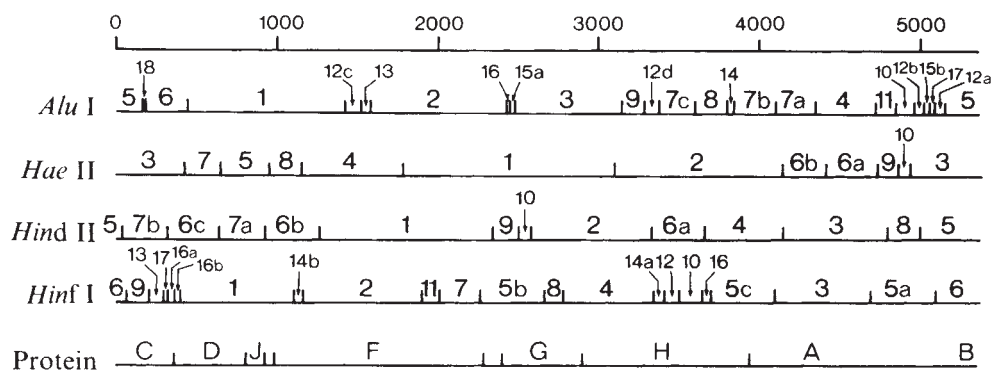


Fig. 1 Restriction endonuclease fragment map of Φ X174 *am3* RFI DNA. The origin of this map is the *Pst*I cleavage site, the scale being the number of nucleotides from that site. The direction of translation is from left to right. The boundaries of genes A, B and C are not precisely defined. The sequence described in this paper is 111 nucleotides between positions, 5,000 and 5,110 approximately. The data on which the cleavage map is based come from the work of Lee and Sinsheimer²³ (*Hae*III and *Hind*II), Vereijken *et al.*¹² (*Alu*I) and Jeppesen *et al.*⁵ (*Hind*II, *Alu*I and *Hinf*I) except that the correct ordering of *Alu*I fragments A12b, A15b, A17 and A12a results from this sequence determination.

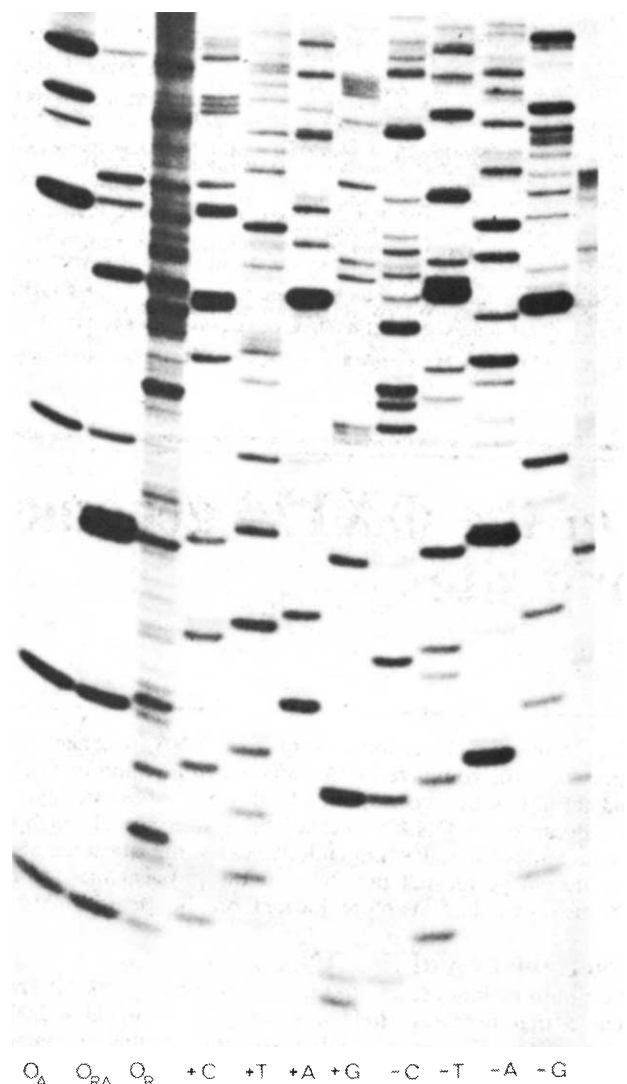


Fig. 2 The 'plus and minus' pattern produced by priming with *Hind*II fragment R8 on complementary DNA template. This defines the sequence from nucleotide 19 to 88 (Fig. 4). The method used to locate the *Alu*I cleavage sites is that previously described for *Pst*I (ref. 24), except in that conditions were selected such that partial cleavage was obtained with *Alu*I. This allows location of all the *Alu*I sites in this region. The *Alu*I cleavage sites are defined by the two channels to the left of the plus and minus pattern, as bands which appear in channel O_{RA} but not in channel O_A .

correct ordering of *Alu*I fragments 12b, 15b, 17 and 12a for the first time^{5,12}. The *Alu*I cleavage sites were mapped in relation to the plus-minus sequence determination as described in Fig. 2.

The sequence of the duplex DNA together with details of the determination is shown in Fig. 4.

Possible assignment of the ribosome binding site

As Φ X174 mRNAs are in the same sense as the viral strand¹³, and the sequence corresponding to the RNA fragment protected *in vitro* is in the viral strand (nucleotides 33–69), this fragment could represent an *in vivo* ribosome binding site. The sequence implicated in ribosome binding (nucleotides 42–46) is in the expected relationship to an initiation codon (ATG, nucleotides 54–56)¹⁴. The adjacent DNA sequence allows the prediction of the sequence of the N-terminal 21 amino acids of the presumed protein product.

It is not possible unequivocally to assign this ribosome binding site, and the putative protein, to a particular gene. The limits of genes H and D are known in relation to the physical map of Φ X174 (ref. 1 and G. M. Air, A. R. Coulson F. Sanger, personal communication). The region between genes H and D contains genes A, B and C. In addition, a

Table 1 Observed molar yields of depurination products

Primer/template Label	<i>Hinf</i> I 6/viral		<i>Hind</i> II 8/complementary	
	dATP	dGTP	dATP	dGTP
Depurination products				
T	2	1	1	2
C	>3 (4)	1	2	
T ₂		1		
C ₂ T			>3 (5)	>3 (4)
C ₂ T ₂		2	1	
C ₂ T ₃	1		2	
C ₂ T ₄		1	1	
C ₂ T ₅	1			1
T ₃	1			
C ₃ T ₃	1			
C ₃ T ₄	1			
C ₃ T ₅	1	1*		
C ₃ T ₆			1	
C ₄ T ₂				

Only the depurination products predicted from the gel sequence data were observed, and in their predicted molar yields. It was not possible to quantitate the products present in greater than three molar yield, and the predicted yields for these are given in parentheses.

*This depurination product established the identity of nucleotide 97 (A, Fig. 4) which was not identified by gel sequence data.

Determination of the N-terminal amino acid sequence of the B protein and any other likely gene products, and their comparison with the 21 amino acid sequence predicted from the DNA sequence data, should allow unequivocal assignment of this ribosome binding site. Further knowledge of the functions of this region of the genome will probably result from a more extensive DNA sequence determination on either side of the sequence presented here, and from examination of the DNA sequence in genetically defined mutants. The results of these studies are presented in ref. 25.

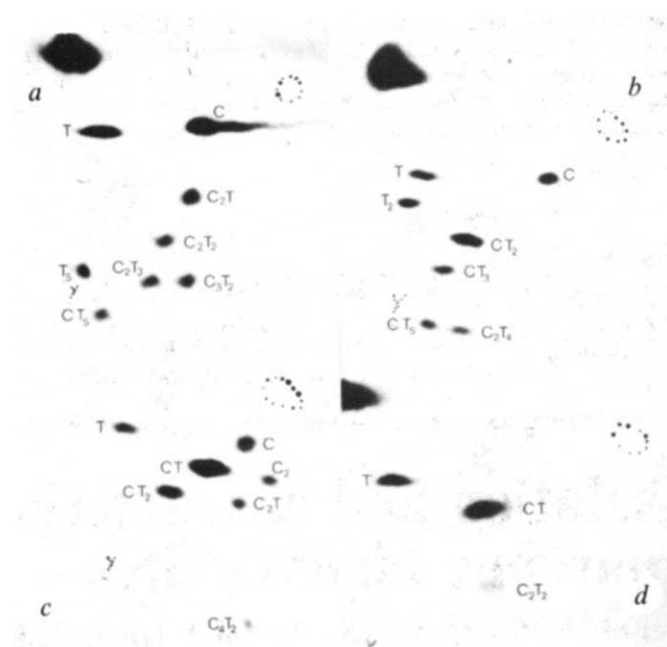
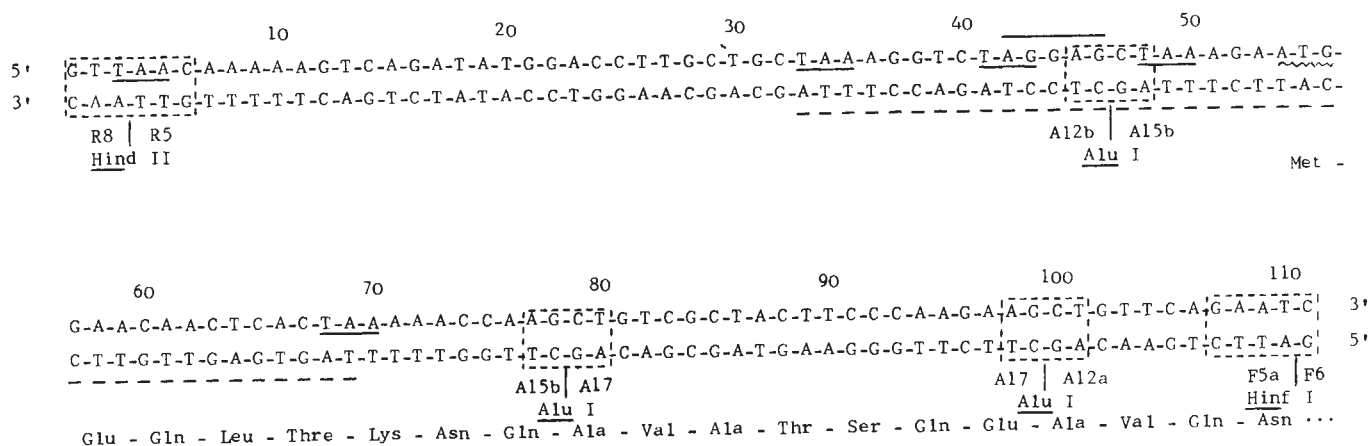


Fig. 3 Depurination analysis. The pyrimidine tracts in each strand were terminally labelled and isolated by the method of Fiddes¹⁰. *Hinf*I fragment 6 was used as a primer on viral strand DNA as template for primed synthesis with either α -³²P-dATP or α -³²P-dGTP label in separate experiments. *Hind*II fragment 8 was similarly used as a primer on complementary strand DNA. The region between the *Hind*II 8/5 and *Hinf*I 5a/6 cleavage sites was isolated by cleavage with both enzymes, followed by electrophoresis of the products on a gel containing 5% acrylamide, 2.5 mM EDTA, 90 mM Tris-borate (pH 8.3). The purified fragment was eluted in 0.5 M NaCl, 0.005 M EDTA, 0.1 M Tris-Cl (pH 8.5) and ethanol-precipitated. Depurination of the fragment and separation of the products were according to the methods of Ling¹¹. As the products were end-labelled by nearest-neighbour transfer of ³²P from the adjacent purine, they could be quantitated by comparison of the relative intensities of the spots on the autoradiogram. The quantitative data is given in Table 1. *a*, *Hinf*I fragment 6, viral strand template, α -³²P-dATP label (that is, complementary strand depurination products). *b*, as (*a*), with α -³²P-dGTP label. *c*, *Hind*II fragment 8, complementary strand template, α -³²P-dATP label (that is, viral strand depurination products). *d*, as (*c*), with α -³²P-dGTP label.

Fig. 4 The sequence of Φ X174 *am3* RF DNA from the *Hind*III 8/5 cleavage site to the *Hinf*I 5a/6 cleavage site. Plus and minus sequence determination priming with *Hinf*I fragment 6 on viral DNA template gave the sequence from 1 to 94. Priming with *Hind*III fragment 8 on complementary DNA template gave the sequence 19–111. Thus most of the sequence is confirmed by complementary data, including the region corresponding to the ribosome protected RNA fragment (33–69; – –). Nucleotide 97 (A) was established solely by depurination analysis. Termination codons are underlined, and restriction endonuclease cleavage sites are boxed. The presumed initiation codon is underlined with a wavy line. The sequence implicated in ribosome binding (AGGAG) is overlined.



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Isolation and characterisation of the Φ X174 ribosome binding sites

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Sequence information for four RNA ribosome binding sites has been obtained from Φ X174 Φ RNA synthesised in vitro. The ribosome binding sites have been assigned to sites on the Φ X174 genome by comparison with known DNA sequence data.

Φ X174 is a single-stranded DNA bacteriophage with a genome of approximately 5,400 nucleotides which codes for nine products (for a review see ref. 1). In the infected cell the mRNA is transcribed from a double-stranded DNA replicative form and translated into the phage proteins. *In vivo*, the proteins made in largest amount are the products of genes *D*, *F* and *G* (refs 2 and 3). Robertson *et al.*^{4–6} isolated a Φ X174 DNA fragment protected by ribosomes which contained a DNA sequence coding for the amino terminal portion of the gene *G* protein. In this laboratory, Pieczenik *et al.*⁷ isolated and determined the sequences of three ribosome binding sites from the mRNA of bacteriophage f1 using techniques that have previously been

shown to yield authentic protein initiation sites from the RNA bacteriophages^{8–10}.

We now have isolated and obtained sequence information for four RNA ribosome binding sites derived from Φ X174 mRNA synthesised *in vitro*. In an accompanying paper, Brown and Smith¹¹ report the DNA sequence of a Φ X174 region which includes one of these sites and thereby locates this site on the Φ X174 genome. Recently, Barrell *et al.*¹² have reported the DNA sequences encoding the amino termini of Φ X174 proteins D, J, F, G and H. Comparison of the DNA and the amino acid sequence data for these Φ X174 proteins allowed them to make these assignments.

Availability of these and other DNA sequence data^{4–6,12} has allowed us to assign the other three ribosome binding sites to Φ X174 genes and to propose complete sequences for them. Our studies of the interaction of Φ X174 mRNA with ribosomes complement the DNA sequence data and allow us to define regions of the genome encoding signals for initiation of protein synthesis.

Fig. 1 Two-dimensional RNase T₁ fingerprint analysis of Φ X174 RNA transcripts before (a) and after (b) ribosome binding. c, Major oligonucleotides of the four ribosome binding sites (I, II, III and IV). Sequence analysis of these oligonucleotides is found in the text and Figs 3, 4 and 5. mRNA transcripts were prepared from Φ X174 RFI and in the experiment shown α -³²P-ATP at 130 Ci mmol⁻¹ was used to prepare the material analysed here. Conditions of transcription and purification of transcripts are as described previously²¹. Ribosome binding was done as described by Steitz⁹ and the fragments isolated and purified as described by Pieczenik *et al.*⁷ Two-dimensional fingerprint analysis was carried out according to Barrell²². The origin is located in the lower right corner of the autoradiograph with electrophoresis at pH 3.5 on cellulose acetate proceeding from right to left and homochromatography from bottom to top on DEAE thin-layer plates.

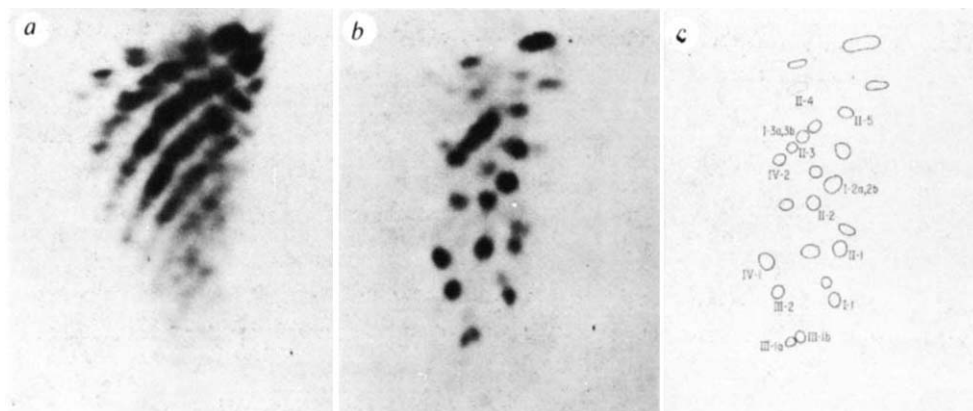


Figure 1 consists of eight panels (a-h) showing fluorescence micrographs of Drosophila ommatidia. Panels a, c, e, and g show normal ommatidia with distinct pigment cells. Panels b, d, f, and h show ommatidia with various defects, including missing or mispositioned pigment cells, labeled with Roman numerals and letters (e.g., I-1, I-2a, I-3b, I-4, I-5, I-B1, I-B2, I-C, I-E, I-F, I-D).

To obtain homogeneous ribosome-protected fragments for further sequence analysis, the mixture of fragments was fractionated using homochromatography as the second dimension of a two-dimensional system¹³, yielding two purified ribosome binding site species (data not shown). Figure 2 shows RNase T₁ and pancreatic RNase fingerprinting analysis of ribosome binding site I (*a-d*) and ribosome binding site II (*e-h*). Experiments were carried out with mRNA labelled by each of the four α -³²P-ribonucleoside triphosphates individually, or labelled by all four triphosphates. Nearest neighbour sequence analysis was exploited to generate the sequence of each oligonucleotide, as well as to determine the order of several of the oligonucleotides⁷.

Panc I	E G	B ₂	F G	B ₁	D G	A G	C	D	G F	E G
	CUAAAGGUCUAGGAGCUAAAGAAUGGAACAACUCACU(A)									
T ₁	2a 5 3a 5 4 2b 3b 5 1									
PROPOSED SEQUENCE	NTP	COMPOSITION	PANCREATIC RNase PRODUCTS	RNase U ₂ PRODUCTS	PROPOSED SEQUENCE	NTP	COMPOSITION	RNase T ₁ PRODUCTS		
1	A	2C, 2A, 1U	A*AC, A*AC* [†] C*U*	CUC*A, C*ACU* [†] A*A, A*	A	A	3A, 1G	A*A*AC*, A*AU		
AACAACUCACU(A)	U	C	AAC*, AC*	C*UCA, C*U	U	U	A	AA*U		
	C	C	AA*G, A*C, U*	CU*CA*, AA*, A*	C	C	A	-		
	A, C	N. D.	AAC, AC, U, C	N. D.	A, C	N. D.	A*AA*G, AAU*	A*AA*AC*, A*AU		
2a	A, U, C, G	A, C, U	A*AA*AG, U*	CU*AA*ACU* [†] A*AA, A*	A	A	G	G*		
	U	2A, 1U	C*	C*UA	U	U	C	C*		
	C	C	-	-	C	C	G	AG*		
CUAAAG(G)	G	A, G	AAA*G*	N. D.	G	2A, G	A*G*, A*G	-		
	A, C	N. D.	A*AA*AG, U*	N. D.	A	N. D.	AG*, G*	A*AA*AG		
	A, U, C, G	C, A, G, U	AAAG, C, U	N. D.	U	G	U	G*		
2b	A	2A, 1G, 1U	A*AA*AG*, U*	CU*AA*ACU* [†] A*AA, A*G*	C	C	U	U		
CUAAAG(A)	U	C	C*	C*UA	G	A	AAA*G*	A*AA*AG, U*		
	C	-	-	-	A, C	N. D.	A*AA*AC, U*	-		
	A, C	A	AAA*G	AA*, A*	A	G, A, C	G*, A*AC*	-		
3a	A, U, C, G	C, A, G, U	A*AA*AG*, U	N. D.	U	C	A	AA*G		
	U	U	U*	UCU* [†] A	C	A	G	G*		
	C	C	U*	U*CUA	A, C	N. D.	AA*AC*, G*	A*AC		
UCUAG(G)	U	A	A*AC*	UCUA*	U	C	A	AA*G		
	A, U, C, G	C, A, G, U	AC, C, U	N. D.	G	A	-	-		
	A	A	A*AU*	A*AA, A*	A, C	N. D.	A*AA*G	-		
3b	U	A	AA*U*	AA*, A*	A	-	-	-		
AAUG(G)	C	-	-	-	C	U	AC*	-		
	G	C, U	AAU*, G*	U*G*	A	A	A*AC	-		
	A, U, C, G	A, C, U	AAU, G	N. D.	A, C	N. D.	A*AC	-		
4	A	-	-	-	A	-	-	-		
	U	-	-	-	C	N. D.	A*G	-		
	C	G	AG*	AG*, G* [†]	A	C	C	-		
AG(C)	G	A	A*G	A*G, A* [†]	U	C	C	-		
	A, C	N. D.	AG	N. D.	C	C	-	-		
	A, U, C, C	A, C	AG	N. D.	A, C	N. D.	C	-		
5	A	G	G	G	A	U	U*	U*		
	U	G	G	G	C	U	-	-		
	C	-	-	-	U	U	-	-		
G(U/A)	A, C	N. D.	G	N. D.	C	-	-	-		
	A, U, C, G	G	G	N. D.	A, C	N. D.	U	-		

Panc					A B G E F H G D E G H C G									
III					GAGGAUAAAUUAUGUCUAAUAUCAAACUG(G)									
T ₁					4 3 4 2 1									
PROPOSED SEQUENCE		NTP	COMPOSITION	PANCREATIC RNase PRODUCTS		RNase U ₂ PRODUCTS		PROPOSED SEQUENCE		NTP	COMPOSITION	RNase T ₁ PRODUCTS		
1		A	3A, 2U, 1C	A*AA*AC, A*AAU*, C*, U*		UCU*AA*, UUC*AA*, U*AA, A*AA*		A		A	G, U	AU*, G*		
UCUAAUAUCAAACUG(G)		C	N.D.	N.D.		N.D.		G		A	A, G	A*G, G*		
		C	2U, 1A	AAA*AC, U*		U*CUA, UU*CA, AA*, A*		U		A	A	A*U		
		U	2C, 2A, 1U	AAAC*, AA*U, A*U*, C*		UC*UA, U*UCA, C*UG		A ₂ C		N.D.	A	AU*, G*		
AUAUAUUAUG(U)		A ₂ C	N.D.	A*AA*AC, A*AAU*, U*, C*		N.D.		A		A	U	A*AA*AU		
		A	A, U	A*AA*AU, U*, U*		UU*AA, A*AA, U*AA*		U		A, U	A, U	AAA*U*		
		C	-	AU*		U*G		A ₂ C		N.D.	A	A*AA*AU		
3		C	-	---		---		C		A	A	A*AA*AC		
		U	3A, 1U, 1G	AAA*U*, A*U, G*		U*UA, UC*, AA*, A*		U		A	A, U	AA*U*		
		A ₂ C	N.D.	A*AA*AU, AU*, U*		N.D.		U		A, U	A, U	A*AA*U*		
AG(G)		A	-	A*G*		A*G*, A*, G*		AU(U/G)		U	A	A*U*		
		C	G, A	-		-		U		A, U	A, U	A*U*		
		U	-	-		-		A ₂ C		C	U	U*		
4		A ₂ C	-	-		-		GU(C)		U	G	G*		
		G	G	G*		G*		G		A	U	U*		
		C	-	-		-		U(A/G/C)		C	U	U*		
G(A)		U	-	-		-		H		A	C	C*		
		A ₂ C	-	-		-		C(A/U)		U	C	C*		

Fig. 5 Sequence analysis of binding sites III and IV. Details of the sequence analysis are described in the text and the legend to Fig. 3. †GAGGAU and AGGAGU were isolated as a mixture and their individual sequences deduced by nearest neighbour sequence analysis.

Panc

IV

T₁

A

AGGAGUUAUUAUGUUUCAG(A)

3 4 3 1 2

PROPOSED SEQUENCE	NTP	COMPOSITION	PANCREATIC RNase PRODUCTS	RNase U ₂ PRODUCTS
1	A	A, U, C	A*AU, U*, C*	UUU*AA*, UUU*AA*, UC*AA
UUUUAUUAUG(U)	G	N.D.	N.D.	N.D.
	C	U	AAU*	U*U*AA*
	U	U, A, G	AAU*, A*U*, U*, G*	U*U*UAAA*, U*U*UA, UG*
	A, C	N.D.	A*AU*, C*, U*	N.D.
	A ₂ C, C, U	A, G, C, U	AAU, AU, C, C, U	UUUAA, UUUA, UG
2	A	C, G	AG*, C*	UUUC*AA
UUUCAG(A)	G	N.D.	N.D.	N.D.
	C	N.D.	N.D.	N.D.
	U	U	U*	U*U*UCA
	A ₂ C, C, U	A, G, C, U	AG, C, U	UUUCA, G
3	A	-	-	-
AG(G/U)	G	A, G	A*G*	A*, G*
	C	-	-	-
	U	G	AG*	G*
4	A	-	G*	G*
G(A)	G	-	-	-
	C	-	-	-
	U	-	-	-
		-	-	-

PROPOSED SEQUENCE	NTP	COMPOSITION	RNase T ₁ PRODUCTS
A	A	G	G*
AGGAGU(U) [†]	G	A, G	A*G*, A*G
	C	-	-
	U	G, U	AG*, U*

is not required to form stable initiation complexes *in vitro*¹⁹. Indeed, in all ribosome binding sites sequenced to date, such potential complementarity to the 3'-end of 16S rRNA has been found to exist. The four ribosome binding sites reported here all possess sequences that have the potential to form base-paired complexes with the 3'-end of 16S rRNA. Thus, the sequence 5'... AGGAG... 3' present in site I has the potential to form five such base pairs, site II can form a possible ten base-paired complex (if a single base is looped out of the 3'-end of 16S rRNA), while sites III and IV can each form a possible five base pairs. The same base-pairing schemes for the DNA sequences encoding sites II, III and IV have been proposed by Barrell *et al.*¹² along with similar schemes for genes *D*, *E* and *H*. We can find no correlation between the number of potential Shine-Dalgarno base pairs seen in an initiation site and the efficiency of ribosome binding and protection *in vitro*. Clearly, other factors must determine which of the protein initiation sites are selected and recognised by ribosomes in our *in vitro* system.

Brown and Smith¹¹, in an accompanying paper present evidence that site I may be the gene *B* ribosome binding site. Their data suggest that the gene *B* protein is encoded by the DNA sequence which also codes for the carboxy-terminus of the gene *A* protein. Barrell *et al.*¹² have shown that gene *E* is similarly coded for by the DNA sequence which also codes for the gene *D* protein. We have found no evidence for a ribosome binding site *in vitro* for either gene *D* or gene *E*. Thus the transcripts from one of the 'included' genes are efficiently protected by ribosomes while those from the other are not.

Binding site II, the proposed gene *J* binding site, contains the sequence 5'-GGAAGGAGUGAUGUAAUG... 3'. The indicated UGA codon would terminate the proposed gene *E* protein¹², whereas the indicated *D* termination codon (UAA) overlaps the indicated gene *J* initiation codon (AUG) by a single base. Platt and Yanofsky²⁰ have observed a similar one-base overlap between the termination of one gene and the initiation of a second gene in the tryptophan operon. Here two termination signals and one functional initiation signal are

compressed within ten bases showing that large intergenic spaces are not required to separate signals for gene expression.

Homologies between published ribosome binding site sequences have been reported. Here we find that sites III (gene *F*) and IV (gene *G*) have a seven out of eight base homology to the left of and including the AUG. In addition, site IV (gene *G*) has six bases (UUUAAU) in common with f1 binding site 1 (gene *VIII*) (ref. 7) and nine out of ten bases from the same region in common with the Q β coat protein ribosome binding site¹⁰. Further analysis will be required to assess the significance of these homologies.

Finally, it should be noted that site IV (gene *G*) is considerably smaller when protected as an mRNA species in a ribosome binding complex than when the single-stranded viral DNA is used in the *in vitro* binding system. Robertson *et al.*⁴⁻⁶ sequenced a 49-nucleotide protected fragment. We find the fragment which is identified as the gene *G* ribosome binding site has a sequence of only 21 nucleotides. The 14 nucleotide-long

I	5'GCUAAAGGUCUAGGAGCUAAAGAAUGGAACAACUCACU(A)3'	(Met—Glu—Gln—Leu—Thr)
II	5'GGAAGGAGUGAUGUAAUGUCUAAAGGUAAAAACG(U)3'	(Met—Ser—Lys—Gly—Lys—Lys—Arg)
III	5'GAGGAUAAAUUAUGUCUAAUAUCAAACUG(G)3'	(Met—Ser—Asp—Leu—Gln—Thr)
IV	5'AGGAGUUUUAUCAUGUUUCAG(A)3'	(Met—Phe—Gln)

Fig. 6 Proposed sequences of the four RNA ribosome binding sites of Φ X174. Details of the sequence analysis are described in the text and Figs 3, 4 and 5. The nucleotide in parentheses at the 3'-end of each sequence represents the nearest neighbour nucleotide determined for the 3'-terminal RNase T₁ digestion product in each case. Binding site I predicts an amino terminal sequence for the gene *B* protein (see text and the accompanying paper). Binding sites II, III and IV specify the amino termini of genes *J*, *F* and *G* (refs 12 and 14–16).

T₁ oligonucleotide on the 3'-end of the DNA sequence was not recovered, nor was a seven nucleotide-long T₁ oligonucleotide on the 5'-end of the DNA sequence. Both of these T₁ oligonucleotides contain extensive polypyrimidine tracts which might explain the poor recovery of these oligonucleotides in the conditions of pancreatic RNase digestion used here to isolate ribosome protected fragments.

We have carefully compared the sequences of these four ribosome binding sites with the six potential ribosome binding sites described by Barrell *et al.*¹² but are as yet unable to explain why only four are recovered in this *in vitro* system. Those which are recovered allow us to define, in conjunction with the accompanying paper and earlier studies^{1-6,12}, additional functional regions of the Φ X174 DNA sequence.

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DNA sequence at the C termini of the overlapping genes *A* and *B* in bacteriophage Φ X174

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Sequence determination of mutants in genes A and B confirms that these constitute another pair of genes in Φ X174 which are translated from the same DNA sequence using different reading frames.

BACTERIOPHAGE Φ X174 is known to code for nine proteins in its DNA (ref. 1) which is about 5,400 nucleotides in length (Fig. 1). The locations of the boundaries of six of the genes—*D*, *E*, *J*, *F*, *G* and *H*—have been defined by sequence determination (F.S. *et al.*, in preparation). Two of these genes (*D* and *E*) overlap on the DNA sequence². To define the boundaries for genes *A*, *B*

and *C* which lie in the remaining region between gene *H* and gene *D*, the sequence of Φ X174 *am3* DNA in the region between the *Hae*III 6a/6b and the *Taq*I 2/7 sites (Fig. 1) has been determined (N.L.B. and M.S., in preparation). A newly recognised ribosome binding site³ has been located in the sequence⁴. The sequence determination of a *B* gene mutant—*am16*—strongly suggested that the *A* and *B* genes are translated from the same DNA using different reading frames (N.L.B. and M.S., in preparation). We now report the 200 nucleotide sequence of Φ X174 *am3* DNA from the *Taq*I 2/7 to the *Hha*I 8b/4 sites (Fig. 1). In addition, the sequences of three mutants from this region—*am18* and *am35* (gene *A*) and a revertant of *am18*, *ts116* (gene *B*)—have been determined. The mutants *am18* and *am35* have an

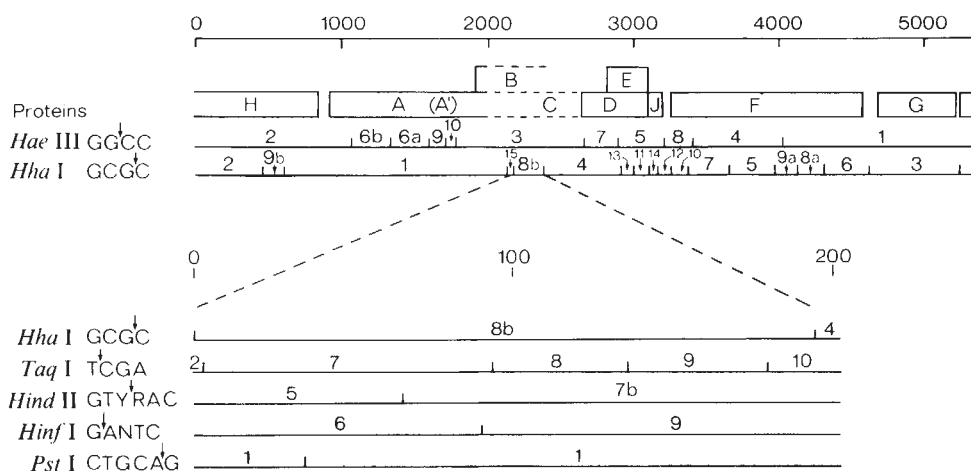


Fig. 1 Map of the Φ X174 genome showing the *Hae*III and *Hha*I fragments and the protein-coding sequences and details of other restriction cleavages within *Hha* fragment 8b.

amber codon at the same site. The gene *B* mutant, *ts116*, results from a single base change in the termination codon of *am18/35*. These results confirm the overlap of genes *A* and *B* and establish the C-termini of the two genes.

Nucleotide sequence analysis

The cleavage sites of five restriction enzymes which cleave in the region are shown in Fig. 1. The sequence of the DNA was obtained by a slight modification of the 'plus and minus' method (ref. 5 and N.L.B. and M.S., in preparation) by priming from the *Hinf*I 6/9 site, on both viral and complementary templates and from the *Pst*I site on the complementary template (Fig. 2). The sequence was confirmed by depurination analysis⁶ of *Taq*I fragments 7, 8, 9 and 10 labelled by 'nick translation'⁷ of Φ X174 *am3* RFL DNA.

Nucleotide sequence of *A* and *B* mutants

Gene *A* mutants (*am18*, *am35*) have been mapped⁸ between the left end of *Hha*I fragment 8b (fragment 7b in ref. 8) and the right end of *Hind*II fragment 5. The mutant *am18* and its revertant *ts116* do not recombine to product *wt* progeny at a frequency above background. These experiments set an upper limit of approximately three nucleotides on the distance separating these mutants⁹. The *B* gene mutant (*ts116*) maps close to, or in, the *Hind*II 5/7b site¹⁰. Consequently, the mutant sequences can be determined very easily using the plus and minus method by priming from the *Hinf*I 6/9 site on the mutant viral strand template. The results of these determinations for *am3* (*E* gene

mutant; wild type in this region), *am18*, *am35* and *ts116* are shown in Fig. 3. The independently isolated *A* gene mutants, *am18* and *am35*, show the same altered nucleotide sequence. In the viral strand, this is a C → T transition at nucleotide 64 (Fig. 2) which results in a CAG → TAG change producing an amber codon in the reading frame which has been assigned to gene *A* (N.L.B. and M.S., in preparation). No other alterations in the *am18* or *am35* sequences can be seen between the *Hind*II 5/7b site and the *Pst*I site (Fig. 2). This transition also results in an amino acid change (Ala → Val) in the reading frame assigned to gene *B* on the basis of the phasing of the ribosome binding site^{3,4} and the gene *B* mutant, *am16*. This assignment of the *am18*, *am35* (gene *A*) mutation to a specific site to the right of the *am16* (gene *B*) as originally suggested by recombination mapping^{1,9,11}, and to a different reading frame, verifies the overlap of genes *A* and *B*.

The gene *B* mutant, *ts116*, was isolated as a revertant of *am18*. The mutation is a G → C change at nucleotide 66, which produces a TAG → TAC (amber → Tyr) codon change in the gene *A* reading frame. In gene *B*, the mutation results in a GAA → CAA (Glu → Gln) codon change. The resultant change in charge may be responsible for the *ts* phenotype.

The original stocks of these *am* mutants were obtained after nitrous acid treatment of viral DNA. The mutations characterised so far include G → A (*am3*,34; *amN11*,27) (ref. 2), C → T (*am18*,35) and G → T (*am16*). This variety of mutations illustrates the caution which should be exercised in predicting the consequences of chemical mutagenesis¹².

Fig. 2 Summary of the plus and minus sequence determination of the *Hha*I fragment 8b region. At the left of each determination is indicated the fragment used as the origin of the plus and minus priming. (The single letter designations used for restriction endonucleases are: *Taq* I (T); *Hinf*I (F); and *Pst*I (P).) Fragments indicated in parentheses are the primers used when these differed from the one corresponding to the origin (for example, F9(T8) indicates that T8 was the primer with the origin at the adjacent *Hinf*I site). The letters V and C indicate that the template was viral or complementary strand DNA, respectively. The designations (S) or (L) indicate the use of a short (4 h) or long (8 h) gel electrophoresis run.

F9(T8)/V(S)	A-T-G-C-G-C-G-C-T-T-C-G-A-T-A-A-A-A-T-G-A-T-T-G-G-C-G-T-A-T-C-C-A-A-C-C-T-G-C-A-G-A-G-T-T-T-A-T-C-G-C-T-T-C-A-T-G-	
P1(T2)/C(A)	C-A-T-G-	
	A-T-G-C-G-C-G-C-T-T-C-G-A-T-A-A-A-A-T-G-A-T-T-G-G-C-G-T-A-T-C-C-A-A-C-C-T-G-C-A-G-A-G-T-T-T-A-T-C-G-C-T-T-C-A-T-G-	
	<u>Hha</u> I 15 <u>8b</u> 21 <u>7</u> <u>Taq</u> I 10 20 30 40 <u>Pst</u> I 11 50 60	
T9/V(S)	T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-	
F9(T8)/V(S)	A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-*T-T-T-C	
P1(T2)/C(S)	A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-C-T-T-T-C-G-G-A-T-A-T-T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-A-T-T	
P1(T2)/C(L)	A-T-G-A-G-T-C-G-A-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-	
	A-C-G-C-A-G-A-A-G-T-T-A-A-C-A-C-T-T-T-C-G-G-A-T-A-T-T-C-T-G-A-T-G-A-G-T-C-G-A-A-A-T-T-A-T-C-T-T-G-A-T-A-A-A-G-C-A-	
	<u>Hind</u> II 5 7b 70 80 90 <u>Hinf</u> I 6 9 7 8 <u>Taq</u> I 100 110 120	
T9/V(S)	G-G-A-A-T-T-A-C-T-A-C-T-T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A	
T10/V(S)	T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-A-C-T-G-C-T-G-G-C-G-G-A-A-A-T-G-A-G-A-A-	
P1(T2)/C(L)	G-G-A-A-T-T-A-C-T-A-C-T-G-C	
F6/C(S)	G-G-A-A-T-T-A-C-T-A-C-T-*C-T-T-G-*-*T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-G	
F6/C(L)	G-C-T-G-G-C-*G-A-A-A-T-G-A-G-A-A-	
	G-G-A-A-T-T-A-C-T-A-C-T-G-C-T-T-G-T-T-T-A-C-G-A-A-T-T-A-A-A-T-C-G-A-A-G-T-G-G-A-C-T-G-C-T-T-G-G-C-G-G-A-A-A-T-G-A-G-A-A-	
	<u>Taq</u> I 8 9 130 140 150 160 170 180	
T10/V(S)	A-A-T-T-C	
F6/C(L)	A-A-T-T-C-G-A-C-C-T-A-T-C-C-T-T-G-C-G-C	
	A-A-T-T-C-G-A-C-C-T-A-T-C-C-T-T-G-C-G-C	
	<u>Taq</u> I 9 10 <u>Hha</u> I 8b 4 190	

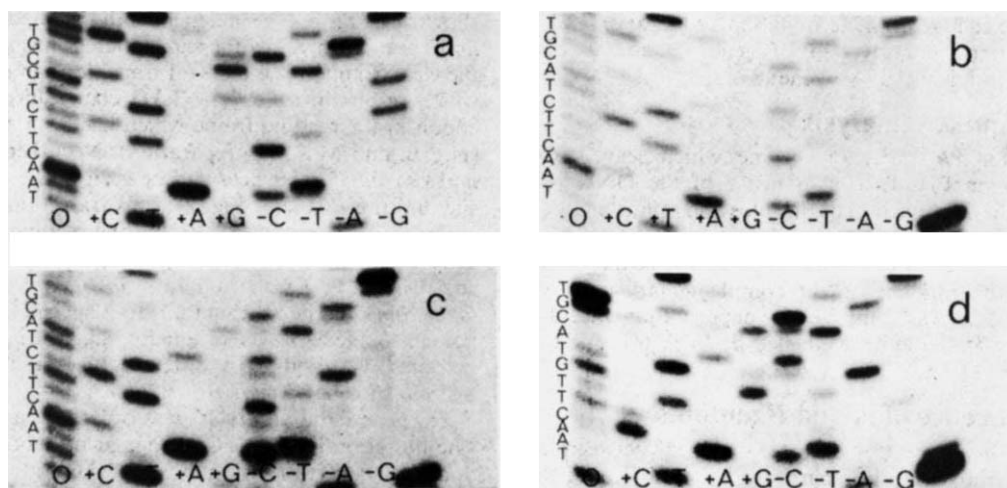
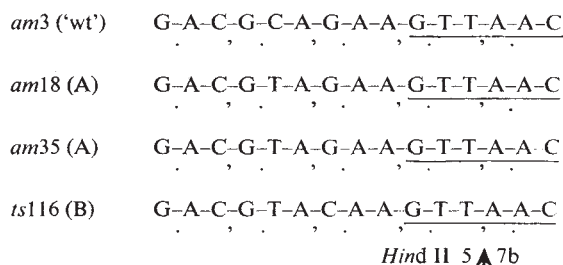


Fig. 3 Sequences of the *am18*, *am35* and *ts116* mutations. The *am3* (wild type in this region) sequence is also shown. *TaqI* fragment 8 was used as primer with *HinfI* as the datum restriction endonuclease with either (a) *am3*, (b) *am18*, (c) *am35* or (d) *ts116* viral DNA as template. The same portion of the autoradiogram of each plus and minus determination is shown with the sequence of the complementary strand deduced. The viral strand sequences, positions 60–74 (Fig. 2), are therefore:



Dots indicate the reading frame for gene *A* and commas that for gene *B*.

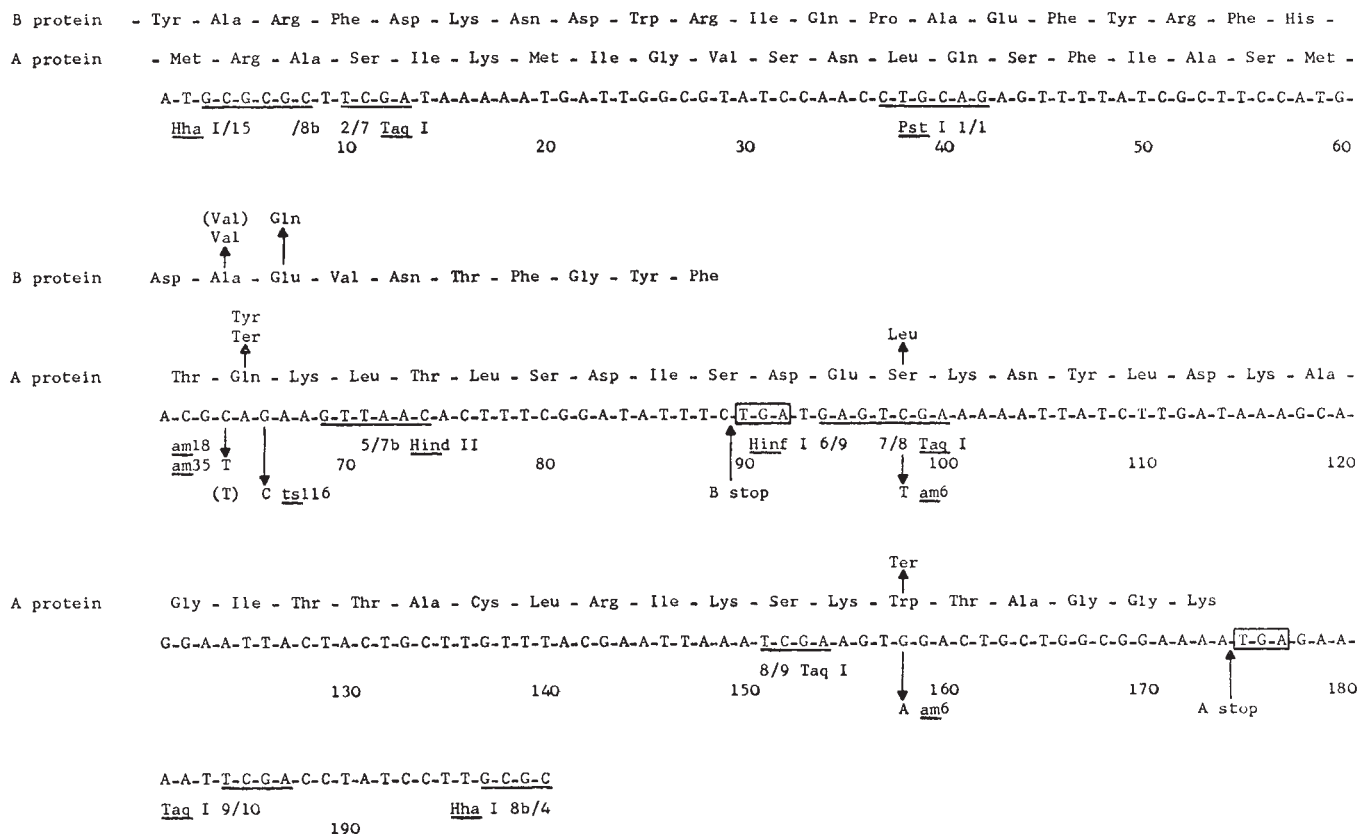


Fig. 4 The sequence of ΦX174 viral DNA in the region of gene *B* and the C terminus of gene *A*. The amino acid sequences of the gene *A* and gene *B* proteins predicted from the DNA sequence are shown together with the changes resulting from the mutations *am18* and the subsequent *ts116*, *am35* and *am6*.

The changes induced in the second gene by mutation in its overlapping companion are not necessarily restrictive, as evidenced by the Leu → Phe change in gene *A* which accompanies the *am16* mutation in gene *B*, the Ala → Val change in gene *B* accompanying the *am18,35* mutation in gene *A*, and the reversion of *am18*, which by two steps induces a Gln → Tyr in gene *A*. There is no evidence for any impairment in protein function due to these changes, whereas in *ts116* the second amino acid change (Glu → Gln) in gene *B* which follows the initial change (Ala → Val) induced by the gene *A* amber mutation evinced a phenotypic response (*ts*).

C termini of A and B proteins

The sequence determination and establishment of the *A* gene reading frame taken together with the *B* gene reading frame allow the prediction of the C termini of the two proteins (Fig. 4). The B protein is 120 amino acids long with a molecular weight of 13,845. The *A* gene extends for 85 nucleotides (28 codons) beyond the C terminus of gene *B*. If, as has been suggested (N.L.B. and M.S., in preparation), the gene *A*/gene *B* overlap arose by read-through of a previously shorter gene *A*, then a question arises about the original role of the DNA beyond the C terminus of gene *B*. The mutant *am6* is a double amber mutant mapping in fragments F9 (gene *A*) and Z7 (gene *E*) (P. Weisbeek, personal communication). In gene *E*

the mutation is identical to *am3*. The gene *A* mutation has also been sequenced and is a G → A change at nucleotide 158. This gives an amber (TAG) codon, and would result in a gene *A* product six amino acids shorter than in the wild type. In addition there is a C → T change at nucleotide 98 which would result in a missense Ser → Leu in the gene *A* protein, and preliminary results suggest yet another change in this region. The resulting gene product is impaired in function, indicating that there is some requirement for the region of the A protein coded by the DNA beyond the end of gene *B*.

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Photochemical attachment of cyclic AMP binding protein(s) to the nuclear genome

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The nature of the cyclic AMP–receptor–nucleus interactions was examined by a novel combination of two photoreactions. A photosensitive derivative of cyclic AMP, N⁶-butyryl cyclic AMP, was covalently attached to its cytoplasmic receptor by photo-affinity labelling and this receptor complex was photo-crosslinked by the DNA in the rat liver nuclei. The photolytic reactions seemed to be specific since stable links were formed only when substantial noncovalent binding occurred.

CLOSE similarity between two modes of signalling to the nucleus in mammalian cell regulation, one by steroid hormones^{1–5} and the other by cyclic nucleotides^{6–10}, has been suggested. Both steroid hormones and cyclic AMP combine specifically with a cytoplasmic receptor protein, and then the resulting ligand–receptor complex enters into the nucleus, where it binds to the genome. Little is known of the nature of these hormone–receptor–genome interactions. A requirement for the solution of this problem is the identification of the residues involved in the protein–DNA interactions in the nucleus.

I intend to study the chemistry of the ligand–receptor and receptor genome interactions and to freeze the *in situ* interacting residues by the covalent attachment of the ligand to the receptor and the receptor complex to the genome in the intact nucleus, so that these residues can be

identified. I report here the combined use of two well-known photolabelling techniques. In one, photo-affinity labelling^{11–13}, the ligand derivative is covalently attached to the receptor protein to identify the ligand binding site. In the other, the photo-induced crosslinking reaction^{14–19}, the protein and nucleic acid are linked together to locate the segments of the macromolecules that are in close contact. This method of 'photo-affinity crosslinking' is based on the ability of the mobile cytoplasmic receptor to bind its ligand specifically and then to migrate to the nuclear site, thus delivering a photosensitive probe to form a ternary nuclear complex.

Photo-affinity crosslinking was used to study the covalent attachment of cyclic AMP to its 'receptor' and the crosslinking of the receptor complex to DNA in the intact nucleus. (In this paper the term 'receptor' is used to signify a high affinity cyclic AMP binding site.) The previously described cyclic-AMP–receptor–nucleus *in vitro* system from rat liver was used¹⁰. The N⁶-butyryl derivative of cyclic AMP (NBcAMP)^{20–21} was chosen for photo-affinity labelling because the carbonyl group on the acyl side chain can be electronically excited by irradiation to a chemically reactive species²². This excitation can produce a variety of free radicals from the rupture of the bond in the α position to the carbonyl (α cleavage) or a 1,5 hydrogen shift of the γ -hydrogen (hydrogen abstraction) and leads to the covalent bond formation between the ligand and the receptor^{11,22}.

Preliminary photo-affinity labelling studies of the cytoplasmic receptor with NBcAMP encouraged us to explore the applicability of receptor site labelling in a more complex system, the cell nucleus. It was expected that NBcAMP would bind specifically to its cytoplasmic receptor proteins,

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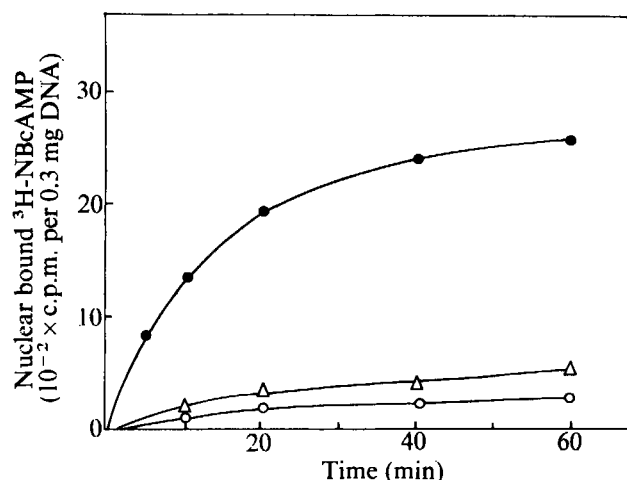


Fig. 1 Time course, temperature dependence and cytosol requirement for the specific uptake of ^3H -NBcAMP by isolated rat liver nuclei. Purified liver nuclei were prepared as before¹⁰. Nuclei (0.30 mg of DNA) were suspended in 0.5 ml of buffer A (0.30 M sucrose, 25 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 3 mM mercaptoethanol, 50 mM Tris-HCl, pH 7.5) and incubated at 4 °C or 25 °C for the indicated times with 0.35 ml of liver cytosol¹⁰ (~ 15 mg of protein ml^{-1} , 105,000g supernatant fraction and equilibrated with buffer B (25 mM KCl, 3 mM mercaptoethanol, 1 mM EDTA, 15% glycerol, 50 mM Tris-HCl, pH 7.5) either by gel filtration through Sephadex G-25 or by dialysis) in the presence of 1×10^{-7} M ^3H -NBcAMP (New England Nuclear). Following incubation, the mixture was chilled, diluted with 2.5 ml cold buffer A, centrifuged (900g, 8 min) and the pellet washed three times with 4 ml of buffer A and finally the nuclear pellet was solubilised (in 0.5 ml of 0.20 M NaOH containing 0.025% SDS). Aliquots were assayed for radioactivity (by liquid scintillation counter) and for DNA (by A_{260}). Each value represents the average of 6 determinations. The specific binding of ^3H -NBcAMP was calculated from the difference in the assay performed in the presence or absence of 500-fold excess of nonradioactive NBcAMP. ●, Incubation at 25 °C with cytosol; △, 25 °C, no cytosol; ○, 4 °C with cytosol.

that this complex, on activation, should migrate into the nucleus to form a nuclear complex, and that photolysis of this nuclear complex should ultimately result in covalent attachment of cyclic AMP derivatives to its receptor and in photochemical crosslinking of the receptor to nuclear DNA. The validity of these predictions was tested.

Nuclear binding of NBcAMP-receptor complex

There is a cytosol requirement for the *in vitro* transfer of cyclic AMP from cytoplasm to nucleus¹⁰. Here I demonstrate, in a similar system, the reversible binding of the NBcAMP-receptor complex to the purified nuclei. When rat liver nuclei were incubated at 25 °C with ^3H NBcAMP in the presence of cytosol, there was time dependent binding of the radioactivity to the nucleus (Fig. 1): in the absence of cytosol, ^3H -NBcAMP bound poorly to the nuclei. Neither heat denatured cytosol, bovine serum albumin (BSA) alone, nor trypsin treated cytosol could stimulate the transfer of ^3H -NBcAMP (Table 1), suggesting that the entire cytoplasmic receptor complex, and not the cyclic nucleotide alone, was transferred to the nucleus.

I have shown that nuclear binding of cyclic AMP, in close similarity to the steroid systems, requires a time, temperature and ligand dependent transformation of the receptor complex, resulting in its increased affinity for the chromatin or for DNA. This was termed the 'activation' step. A similar time and temperature dependence was demonstrated for NBcAMP receptor complex (Fig. 1). At 25 °C, the transfer was nearly complete in 30 min, but at 4 °C the reaction was much slower.

The binding of ^3H -NBcAMP to cytosol receptor seems to be specific since the interaction could be prevented only by the addition of a large excess (400-fold) of non-radioactive cyclic AMP or NBcAMP, but not by excess of ATP, AMP, or GMP (Table 1). The fact that the NBcAMP binding was inhibited by cyclic AMP as well as by NBcAMP suggests that they both interacted with the same receptor site^{10,21}.

The nuclear binding of the receptor complex seems to be noncovalent, since the NBcAMP-receptor complex is extractable from the nucleus with 0.4 M KCl. In addition, there is no apparent chemical change in the cyclic nucleotide during the nuclear binding, since at least 90% of the bound radioactivity was shown to be identical with ^3H -NBcAMP on TLC^{10,23}.

Photolytic incorporation of NBcAMP-complex into the nucleus

Preliminary photo-affinity labelling studies of the cytoplasmic receptor with ^3H -NBcAMP (in preparation) en-

Table 1 Cytosol requirement and comparison of non-covalent and covalent nuclear binding of ^3H -NBcAMP

Comparison of non-covalent and covalent binding	Non-covalent nuclear binding	
	(c.p.m. per 0.7 mg of DNA)	TCA precipitated counts after 15 min ultraviolet* (c.p.m.)
Cytosol	5,360	2,170 (140 no UV)
No cytosol	1,040	260
Heat denatured cytosol (15 min at 65 °C)	1,270	310
Trypsin treated cytosol†	1,210	320
Cytosol replaced by bovine serum albumin (12 mg ml^{-1} in buffer B)	1,330	370
Cytosol + nonradioactive cyclic AMP (5×10^{-5} M)	320	140
Cytosol + nonradioactive NBcAMP (5×10^{-5} M)	410	180
Cytosol + AMP (5×10^{-5} M)	5,210	2,230
Cytosol + ATP (5×10^{-5} M)	5,460	2,290
Cytosol + GMP (5×10^{-5} M)	5,370	2,240
Enzymatic digestions of the irradiated ^3H -NBcAMP ternary nuclear complex‡		
DNase	—	2,180
RNase	—	2,220
Trypsin	—	260

Nuclei (0.7 mg of DNA) and cytosol (0.75 ml; 12–15 mg protein per ml) were incubated with 1×10^{-7} M ^3H -NBcAMP for 50 min at 25 °C. After the samples were chilled, centrifuged and washed, the nuclear bound ^3H -NBcAMP was determined as described in Fig. 1.

*Photolysis of the ^3H -NBcAMP nuclear complex (prepared as above) was carried out as described in Fig. 2 for 15 min; aliquots were assayed by TCA precipitable radioactivity.

†Cytosol was treated with trypsin (0.2 mg ml^{-1} , Worthington) for 30 min at 25 °C and then soybean trypsin inhibitor (0.35 mg ml^{-1}) was added for 5 min before incubation with the nuclei.

‡Photolysis of ^3H -NBcAMP ternary nuclear complex was carried out as described in Fig. 2b, followed by enzymatic digestion with DNase (0.1 mg ml^{-1}), RNase (0.1 mg ml^{-1}) and trypsin for 45 min at 30 °C as described in Fig. 3 and finally analysed for TCA insoluble radioactivity as above.

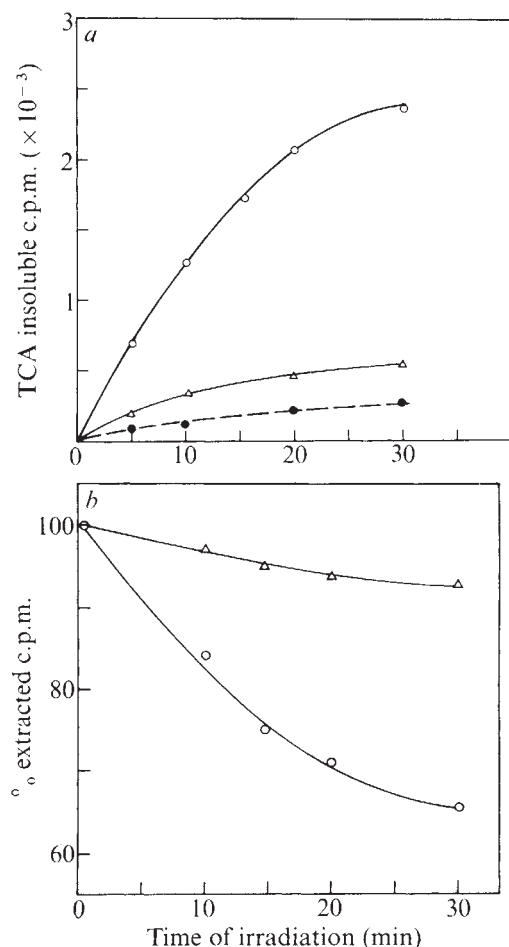


Fig. 2 Time dependence of the photolytic incorporation of ^3H -NBcAMP into the nuclear complex. *a*, TCA precipitable counts; the ^3H -NBcAMP nuclear complex was prepared, as described in Fig. 1, by incubating nuclei (1 mg of DNA) and cytosol (1.2 ml, 12–15 mg of protein ml^{-1}) at 25 °C for 50 min with ^3H -NBcAMP (1×10^{-7} M) and resuspended in 2.5 ml of buffer C (0.20 M sucrose, 25 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 7.5 mM mercaptoethanol, 1 mM EDTA, 20% glycerol, 50 mM Tris-HCl, pH 7.5) and placed into wells of a plastic culture plate on ice, equipped with magnetic stirrer. The reaction mixture was irradiated at a distance about 8 cm with a 25-W mercury lamp (General Electric, predominantly 253.7 nm radiation). At the indicated time, 0.60-ml aliquots of the irradiated mixture were assayed by TCA precipitable radioactivity (2 ml of cold 20% TCA was added to the aliquots, centrifuged and washed 3 times with 4 ml of 5% TCA), the pellet was dissolved in 0.5 ml of 0.20 M NaOH containing 0.025% SDS and assayed for radioactivity. ^3H -NBcAMP-cytoplasmic receptor complex was prepared by incubating cytosol (3 ml) with ^3H -NBcAMP (1×10^{-7} M) for 30 min at 4 °C and equilibrated with buffer B either gel filtration (through Sephadex G-25) or by dialysis, and finally was irradiated as above. Controls were treated identically, but were not exposed to ultraviolet light. Complete nuclear incubation mixture irradiated (○); complete nuclear mixture unirradiated (control) (●); irradiated in the absence of cytosol (Δ). *b*, Percentage of radioactivity released by 0.4 M KCl in buffer C as described above. At indicated times aliquots were removed, KCl concentration uniformly adjusted to 0.4 M, centrifuged, extracted again with 1 ml of 0.4 M KCl (in 50 mM Tris, pH 7.5), and the combined supernatant (nuclear extract) was assayed for the protein bound radioactivity by gel filtration (13 $\times$ 1.25-cm column of Sephadex G-25); 65–75% of the extracted nuclear radioactivity was present in the macromolecular fraction. The amount of released radioactivity from the nuclei is expressed as a percentage of the nonirradiated control. Irradiation in the absence (○) and in the presence (Δ) of 0.4 M KCl.

Table 2 Activation and photochemical fixation

	Salt extracted (c.p.m.) from nuclei (% of control)
Activation at 25 °C (control; no ultraviolet)	100
Activation followed by ultraviolet*	69
Ultraviolet exposure followed by activation†	93
Activation followed by ultraviolet and then the DNase digestion‡	98

Nuclear suspension (0.35 mg DNA) and cytosol (in the same ratio as in Fig. 1*a*) were incubated in buffer C with 1×10^{-7} M ^3H -NBcAMP for 50 min at 25 °C, then incubated again for 20 min at 4 °C (in the irradiation plate, as in Fig. 2, but not exposed to ultraviolet). The nuclear suspension was then extracted with 1 ml of 0.4 M KCl in 50 mM Tris, pH 7.5. After standing for 30 min with occasional agitation, the nuclei were pelleted and the KCl extract containing the ^3H -NBcAMP protein complex was analysed by Sephadex G-25 gel filtration for macromolecular bound radioactivity as described in Fig. 2*c* legend.

*Nuclei and cytosol were incubated as above and the nuclear suspension was irradiated for 20 min at 4 °C, extracted and macromolecular radioactivity was estimated as above. The amount of released radioactivity from the nuclei is expressed as a % of the nonirradiated control.

†Nuclei and cytosol were incubated with ^3H -NBcAMP in buffer C as above, but at 4 °C for 15 min and then irradiated for 20 min at 4 °C. The irradiated suspension was then activated by incubation for 50 min at 25 °C and then extracted and assayed.

‡Nuclei and cytosol were incubated with ^3H -NBcAMP for 50 min at 25 °C, then photolysed for 20 min. After irradiation the samples were treated with pancreatic DNase I (Worthington, final concentration 0.1 mg ml^{-1} in 50 mM Tris, pH 7.5, containing 2 mM CaCl_2 and 2 mM MgCl_2 , incubated for 35 min at 30 °C with occasional shaking and terminated by addition of EDTA, to final concentration of 15 mM). For the DNase control, EDTA was added before the DNase. Extracted radioactivity was then estimated as before (mean of four experiments). The amount of released macromolecular radioactivity in the control was 2120 c.p.m. per 0.35 mg of DNA.

couraged me to explore the receptor labelling in the cell nucleus. Photolysis of the NBcAMP nuclear complex at 254 nm was followed by TCA precipitable counts as a function of time of irradiation. Results of a typical nuclear irradiation experiment (Fig. 2*a*) show that the amount of radioactivity associated with the macromolecules was directly proportional to the time of irradiation. Also, in the absence of irradiation (control), no significant radioactivity was rendered TCA precipitable. The reaction seems to be covalent, as the radioactivity remains associated with the macromolecular fraction during treatments which remove noncovalently bound ligands, such as extensive dialysis against 7 M urea, Sephadex G-25 filtration in the presence of 0.5% SDS, or 10 min boiling with TCA. The photolytic incorporation of ^3H into the nuclear macromolecular component seems to be specific, because the reaction could only be prevented by addition of a large excess of non-radioactive cyclic AMP or NBcAMP (Table 1), with no significant competition with other nucleotides such as AMP, ATP and GTP. Further, the photolytic process could not be stimulated in the absence of cytosol, or with cytosol replaced by heat denatured or trypsin treated cytosol, or serum proteins (Table 1*b*). Thus an intact cytoplasmic protein seems to be required for the photochemical reaction, as well as for the non-covalent nuclear binding of NBcAMP.

When the ^3H -NBcAMP nuclear complex was incubated in the dark and then subjected to extraction in 0.4 M KCl, most of the radioactive receptor complex was recovered in the extract. When the same complex was irradiated, there was a time dependent decrease in the recovery of the complex: this decrease was not seen if the photolysis itself was carried out in the presence of 0.4 M KCl (Fig. 2*b*). About 25% of the radioactive complex can no longer be extracted with salt (as compared with the non-irradiated control), suggesting that a portion of the complex becomes

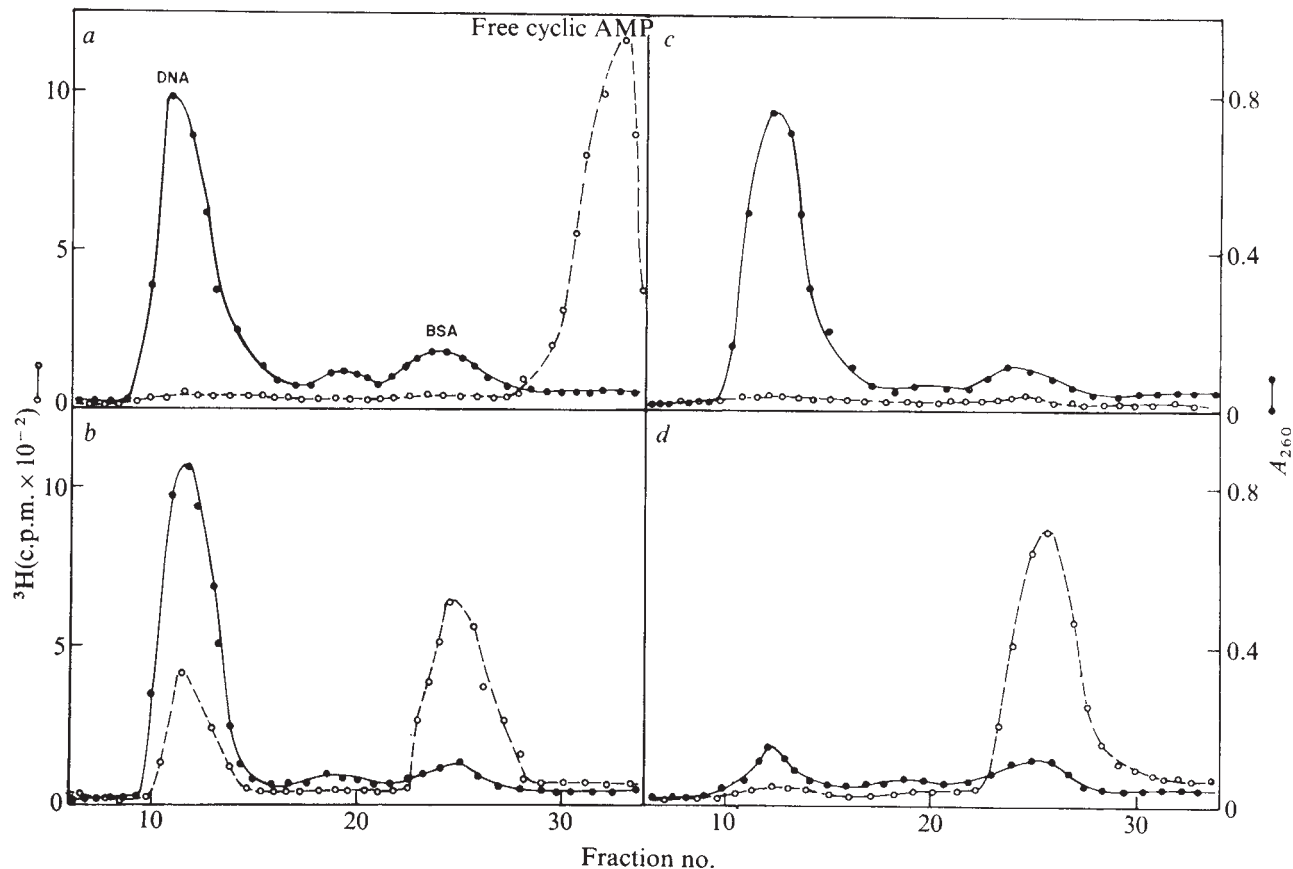


Fig. 3 Characterisation of the irradiated ^3H -NBcAMP nuclear complex by SDS Sepharose 2B chromatography. DNA (calf thymus), protein (BSA) and ^3H -NBcAMP were used as markers and their respective positions are indicated. Photolysis (for 15 min) of ^3H -NBcAMP nuclear complex (1 mg DNA) was carried out as for Fig. 2. In some experiments the noncovalently bound ^3H -NBcAMP was removed by TCA precipitation before solubilisation. The TCA or nuclear pellets were then solubilised in about 1 ml of 1% SDS (containing 0.025 M EDTA, 0.05 M mercaptoethanol, 0.1 M Tris-HCl pH 8) by heating it at 60 °C for 20 min, with occasional shaking and the sample was applied to a Sepharose 2B column (1.3 × 45 cm) equilibrated (at room temperature) with Tris-SDS buffer (0.15 M KCl, 0.01 M EDTA, 0.05 M mercaptoethanol, 0.1 M Tris-HCl pH 8, 0.5% SDS) and 1-ml fractions were collected for determination of absorbance and radioactivity. After irradiation and before solubilisation, all samples were sonicated (to a DNA molecular weight about 5×10^6). *a*, Elution profile of non-irradiated control was treated identically but not exposed to ultraviolet and to TCA. *b*, Profile of the photolysis sample (with TCA precipitation). *c*, Chromatography of the irradiated complex after trypsin digestion. Following the irradiation, samples were subjected to trypsin treatment (Worthington, final concentration 0.1 mg ml⁻¹, incubated at 30 °C for 45 min, and precipitated with 50% of TCA to final concentration of 10%). The precipitates were collected by centrifugation and washed (sequentially with acetone 3 ml and ether 2 ml) and the pellets were finally suspended in 1% SDS, and analysed by SDS Sepharose chromatography as above. *d*, Chromatography of the irradiated complex after DNase digestion. After photolysis the samples were digested with pancreatic DNase 1 (Worthington, final concentration 0.1 mg ml⁻¹ in 50 mM Tris-HCl buffer, pH 7.5, containing 2 mM CaCl₂ and 2 mM MgCl₂, incubated for 40 min at 30 °C with occasional shaking and terminated by TCA precipitation). The digested samples were analysed by SDS Sepharose 2B chromatography. *e*, Irradiation in presence of 0.4 M KCl; ^3H -NBcAMP nuclear complex was suspended in 1 ml of buffer C containing 0.4 M KCl irradiated for 15 min as before and analysed by SDS Sepharose 2B chromatography.

'fixed' in the nucleus after 20 min of irradiation. Alternatively, the receptor site may be destroyed during the irradiation, with the radioactive ligand being somehow retained by the nucleus.

If the ^3H receptor complex is fixed in the nucleus by covalent attachment, then the nature of the linkage could be characterised by enzymatic digestion. Trypsin and pronase digestion of the photolysed nuclear complex rendered most of the radioactivity TCA soluble (Table 1), but treatment with DNase or RNase did not. DNase treatment, however,

resulted in drastic liberation of the fixed ^3H -receptor complex from the nuclei, so that the previously fixed radioactivity became extractable with 0.4 M KCl (Table 2). Digestion with DNase suggests that DNA is involved in the nuclear fixation and that the receptor site was probably not destroyed during the irradiation. These experiments are consistent with (but do not prove) the hypothesis that during photolysis the ligand becomes covalently attached to the cytoplasmic receptor and the receptor complex, in turn, is crosslinked to the nuclear DNA.

Specificity of the photochemical reaction

Is the photochemical reaction specific, or is it simply a random collision of free radicals and/or other active species generated during the irradiation process? If the photo-affinity labelling is specific, the reagent molecule would probably bind reversibly and specifically to the receptor site(s), and then bind covalently after photolysis. Thus, conditions which would interfere with specific reversible binding would also prevent covalent binding. I expected first, that a denatured or degraded receptor which is unable to interact either with ligand or with the nuclei would be unable to mediate the photochemical attachment of ligand to receptor or receptor to nuclei. Second, dissociating conditions in 0.4 M KCl which prevent the reversible nuclear binding of the receptor complex should also prevent the photochemical attachment to the nuclei. Third, absence of the heat activation step should have a similar effect. Thus, if the photochemical reaction is specific, then in the absence of reversible binding, there should be no covalent bound formation at the nuclear site.

The results support these predictions. First, degraded receptor did not promote the covalent photoreactions, or the noncovalent nuclear binding of the receptor complex (Table 1). Second, in the presence of 0.4 M KCl when the receptor complex was not associated with the nucleus (Fig. 2*b*), photolysis yielded only affinity labelling at the receptor site, but the receptor was not attached to the nucleus. Third, to verify the heat activation requirement, experiments were carried out in the reverse sequence: when the activation (binding) step was preceded by irradiation, again no nuclear linked receptor was observed (Table 2). These results are consistent with the implication that the photochemical reactions are specific: stable links are formed only when substantial noncovalent binding occurs.

Characterisation of nuclear photolysis products

The chemical characterisation of the product formed between ^3H -NBcAMP, protein and DNA during irradiation of the nucleus was performed using enzymatic digestions, followed by Sepharose 2B chromatography in the presence of detergent (0.5 SDS) to denature and to dissociate all noncovalently linked molecules. A typical chromatogram of nonirradiated nuclei is shown in Fig. 3*a*. The DNA appeared in the void volume, followed by proteins, and finally by free radioactive ligand. In the presence of the detergent, there was essentially no radioactivity associated with either the DNA or the protein fractions. After 15 min of irradiation, however, there was a striking change in the chromatographic profile (Fig. 3*b*). Two new macromolecular radioactive peaks appeared: the first, in the void volume, coeluted with DNA, and the second, larger peak, appeared with proteins. For the first peak, I have considered the following possible linkages formed during the irradiation between ^3H -NBcAMP (cA) and macromolecules: cA-DNA, cA-DNA-protein, DNA-cA-protein, cA-protein-protein(s). These structural alternatives might be distinguished by specific enzymatic digestions. After DNase digestion (Fig. 3*d*), no radioactivity remained associated with the DNA peak, no additional free radioactive ligand was liberated, and all radioactivity migrated with the protein fraction. In contrast, trypsin treatment (Fig. 3*c*) liberated all macromolecular bound radioactivity. Thus, there is probably a protein 'bridge' to link the ^3H ligand to DNA, for example ^3H -cA-protein-DNA, and there seems to be no direct association between the ligand and DNA.

I have indicated that the dissociating condition prevented both the noncovalent nuclear binding and the

covalent attachment of receptor to the nuclei. To substantiate this conclusion, I analysed the chromatographic profile of the reaction products. When the ^3H -receptor complex and nucleus were irradiated under dissociating conditions (0.4 M KCl), (Fig. 3*e*), radioactivity was associated mainly with the protein fraction, and only minor amounts with the DNA. Furthermore, when the photolysis was carried out before the activation step, there was essentially no radioactivity in the DNA fraction (data not shown). These experiments further demonstrate that covalent attachment is observed only where substantial noncovalent association occurs.

The alternative that the covalent reaction occurs in the nuclei between the NBcAMP and certain proteins, but without the involvement of the cytoplasmic receptors, is unlikely because essentially no covalent photo-reaction occurs in the absence of the cytosol. It remains possible that the ligand is somehow transferred from its original receptor to another nuclear constituent ('pseudo-photo-affinity labelling', ref. 24). If the nuclear crosslinking product did not involve DNA, this would be difficult to reconcile with the DNase sensitivity of the photo-adduct. Finally, the possibility that a crosslinking reaction occurs with a relatively unspecific part of nuclei, such as nonchromatin material, is also unlikely, as, on photolysis, the same type of crosslinking product was obtained when the nuclei was replaced by purified chromatin (Kallos, unpublished), but not with DNase treated nuclei.

Conclusion

The formation of a stable ternary complex, presumably of covalent nature, has been achieved through specific photolysis of the NBcAMP-protein-nuclei system. The exact chemical nature of the stable joint nuclear complex is not yet known. It probably involves the covalent attachment of NBcAMP to the receptor site and the crosslinking formation between reactive DNA base(s) and protein residues that are in close proximity in the ternary complex.

These results suggest that the optimal noncovalent, as well as covalent, nuclear binding of cyclic AMP require the presence of an intact cytoplasmic receptor. I propose the following reaction sequences (scheme 1) for the reversible interactions (sequence a): (1) the cyclic AMP derivative, NBcAMP (cA), binds specifically and reversibly to a soluble cytoplasmic receptor protein (giving cA·R); (2) this is followed by a temperature-dependent (T°) activation step¹⁰ (cA·R'); and (3) there is an association of this activated complex with the nucleus (cA·R'·N). The photolysis of this reversible nuclear complex (step b) results in the covalent link formation (cA*-R*-N*). The asterisks represent the as yet unidentified photochemically altered components. When the receptor complex is not associated with the nucleus; for example, in the presence of 0.4 M KCl or in the absence of activation, photolysis yields only an affinity label at the cyclic AMP site (cA*-R*), but the receptor is not attached to the nucleus.

The function of the receptor protein, possibly the regulatory subunit of a protein kinase, remains to be determined. It is interesting to note that cyclic AMP dependent protein kinases, which can bind to nuclei (or to DNA), have recently been described in the thymus²⁵ and in the ovary²⁶. Although the photochemical reaction was shown to be specific by several criteria, one must be cautious in the interpretation of the results due to the tendency of the receptors to aggregate^{5,27,28}.

The particular value of photo-affinity crosslinking is to provide a chemical tool for structural study of the locus of the genome which interacts with the receptor. The present study can only be considered preliminary until the photoproducts are separated and further identified.

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letters to nature

Cosmogony and the magnitude of the dimensionless gravitational coupling constant

SIMPLE arguments involving gravitational fragmentation indicate that for the Universe to contain galaxies and stable nuclear-burning stars, the dimensionless gravitational coupling constant $\alpha_g (= Gm_p^2/\hbar c)$ must lie within several orders of magnitude of its actual value (5×10^{-39}). The characteristic mass of a galaxy is shown to be $\alpha_g^{-5/2}$ nucleons. If galaxies and galaxy clusters form by gravitational instability and fragmentation of inhomogeneities in the early Universe, the large-scale structure of the Universe imposes a lower bound, and the instability requirement an upper bound (equal to $\alpha_g^{-1/4}$), on the entropy per particle.

Gravity is an exceedingly weak force. A convenient measure of its magnitude is given by the dimensionless constant $\alpha_g = Gm_p^2/\hbar c$ (the ratio of gravitational to electromagnetic forces between a pair of protons multiplied by the atomic fine structure constant $\alpha (= e^2/\hbar c)$, and equal to 5×10^{-39}). The origin and significance of this number have provided a continuing source of speculation among physicists, whose very existence (and indeed the existence of all life) may be contingent on its value^{1,2}.

Certain elementary and speculative considerations show that for the Universe to contain galaxies and nuclear-burning stars, α_g must lie within a few orders of magnitude of its actual value. In outlining these arguments, I shall also show how the magnitude of one of the most fundamental of cosmological constants, namely the entropy per baryon, can be related to the scale of the large-scale structure of the Universe and the value of α_g . Two significant large number coincidences involving α_g that I will not discuss are the total number of baryons in the observable Universe ($N_u \sim \alpha_g^{-2}$) and the age of the Universe ($\sim \alpha_g^{-1/2} \hbar/m_p c^2$) (see refs 1–5).

My discussion is based on some recent work⁶ on the fragmentation of self-gravitating gas clouds. The concept of gravitational instability was originally presented as a quantitative criterion by Jeans, but Hoyle first stressed the fundamental role of dissipation and fragmentation in gravitational collapse⁷. It is convenient to discuss separately the problems of star and galaxy formation.

Consider a collapsing primordial gas cloud, which can initially be neutral and consist of hydrogen and helium. The cloud will heat up until at $\sim 10^4$ K it can cool by excitation of Lyman α -photons, and will continue to collapse approximately isothermally. While the collapsing cloud can radiate freely, it will be

gravitationally unstable to fragmentation on smaller and smaller scales, until eventually the individual fragments become opaque either to electron scattering or to H^- opacity. The subsequent, quasi-adiabatic collapse of what one may now identify as protostellar fragments, will be stable against continued fragmentation, provided that the collapsing fragments individually undergo a luminosity-controlled Kelvin–Helmholtz contraction.

To maintain stability, a fragment must be unable to radiate away freely the compressional energy acquired by collapse. The collapse will proceed with $\gamma \approx 1$, and will be unstable to small perturbations: this should manifest itself, however, as convection or turbulence because the ensuing pressure gradients will have a strong stabilising influence on any tendency to further subfragmentation. Sufficiently massive fragments will, however, undergo free-fall, and in this case fragmentation can continue until smaller fragments form that are themselves pressure-supported. This condition can be expressed quantitatively by requiring that the Kelvin–Helmholtz contraction time of a fragment exceed its free-fall time, when the gas temperature is ~ 0.1 Rydberg (characteristic of hydrogen atom excitations by electron and atom collisions). The Kelvin–Helmholtz contraction time for a protostar of primordial composition, mass M , and luminosity L is $t_{KH} \sim M/(kT/\mu)L^{-1} \sim (k/\mu)^5 T \sigma_T G^{-4} (m_p \sigma)^{-1} M^{-2}$, where μ is the mean molecular weight g^{-1} , σ is the Stefan–Boltzmann constant, σ_T is the Thompson cross-section, and T is the central temperature. Since the free-fall time $t_{ff} \sim (G\rho)^{-1/2} \sim GM/(kT/\mu)^{3/2}$, the condition $t_{KH} > t_{ff}$ is equivalent to $M < (k/\mu)^{13/6} T^{5/6} G^{-5/3} (\sigma_T/m_p \sigma)^{1/3}$. One consequently infers that this is equivalent to imposing an upper limit on the mass of a protostar of $N_p \sim \alpha_g^{7/3} \alpha_g^{-5/3}$ nucleons or about $\sim 50 M_\odot$. For conventional interstellar matter, where the relevant temperature ($\sim 10^{-2}$ Rydberg) is determined by the melting of refractory grains, this limiting mass is approximately $0.2 M_\odot$ ⁸. Thus typical stars forming now should have relatively low mass (although other effects, notably due to magnetic fields, may enable more massive stars to form), whereas primordial stars are likely to be massive stars.

The mass of a stable nuclear-burning star is known^{1–5} to be of order $N_* \sim \alpha_g^{-3/2}$. Apart from numerical factors, less massive stars have degenerate cores, and more massive stars are dynamically unstable. Thus if gravitational fragmentation is to lead to star formation, one evidently requires $N_* \ll N_p$, or $\alpha_g \ll \alpha^{14}$. This upper bound on α_g ($\sim 10^{-30}$) demonstrates the weakness of the gravitational interaction, as determined solely by the requirement that protostellar collapse leads to the formation of stable nuclear-burning stars. If this inequality were violated,

stars may still form (compare ref. 9) but would not have undergone a pressure-supported core accretion phase. Such stars may differ drastically from conventional stars; for example, condensation of planets may be inhibited (compare also ref. 2).

Consider next the collapse of a galaxy from an ionised protogalactic gas cloud of arbitrary size but of some specified mass. Such a cloud must initially collapse in adiabatic free-fall, its cooling time ($\propto \rho^{-1}$) being long compared with its free-fall time ($\propto \rho^{-1/2}$). At the instant when the local cooling rate overtakes the collapse rate, if the cloud is moderately inhomogeneous, it will become unstable to fragmentation, and erupt in star formation. The hydrogen cooling rate, due mostly to free-bound transitions at $kT \lesssim 1$ Rydberg, can be expressed as $t_{\text{cool}}^{-1} \propto \rho T^{-3/2}$; hence the mass of a gas cloud in gravitational equilibrium satisfies $M \propto \rho^{-1/2} T^{3/2} (t_{\text{cool}}/t_{\text{ff}})$. Since fragmentation into stars proceeds at $T \sim 0.1$ Rydberg, clouds more massive than N_{gal} (defined by $t_{\text{cool}} = t_{\text{ff}}$) must form stars at a much slower rate (by eventually cooling to ~ 0.1 Rydberg). This necessarily qualitative argument suggests that there is a critical mass $N_{\text{gal}} \sim \alpha^5 \alpha_g^{-2} (m_p/m_e)^{1/2} (\sim 10^{12} M_\odot)$ below which clouds can cool within a free-fall time: larger, more slowly evolving, clouds cool relatively slowly and are liable to subfragmentation or disruption by collisions with neighbouring clouds. One infers that N_{gal} is a characteristic mass for galaxies. (One can also show that associated with this mass-scale is a characteristic dimension of ~ 50 kpc.) Since the number of baryons in the observable Universe is of the order $N_u \sim \alpha_g^{-2}$ (ref. 3), one infers that the number of observable galaxies should be of the order $N_u/N_{\text{gal}} \sim \alpha^{-5}$, whereas the number of stars in a galaxy is $N_{\text{gal}}/N_* \sim \alpha^5 \alpha_g^{-1/2}$. It is amusing that these numbers only coincide if $\alpha_g \sim \alpha^{-20}$.

Introduction of certain cosmological considerations can further our speculations. At present, the Universe is well described by the Friedmann-Lemaître cosmology, and observations of the microwave background radiation reveal the homogeneity and isotropy of the Universe at early epochs. In such a cosmological framework, primordial curvature fluctuations play an essential role in accounting for large-scale deviations from homogeneity. The scale of initial amplitudes of these fluctuations is not relevant; it suffices to say that the Universe is observed to be lumpy on the scales of galaxies, galaxy clusters and superclusters, and reasonably smooth on much larger scales.

A natural length scale for large-scale structure arising out of the early Universe is the maximum Jeans mass (N_J) which is achieved at the epoch when matter and radiation densities are equal. Shortly thereafter, matter and radiation decouple, and the Jeans mass is drastically decreased. Any disturbances in the radiation era (due to inhomogeneities of finite spatial extent) on scales smaller than N_J will act as a diverging source of waves that propagate out to N_J (in much the same way as water waves are produced by dropping stones into a pond). Neighbouring regions of the Universe similarly develop structure on scale N_J , with the result that structure inevitably develops over the maximum Jeans length in the early Universe (provided one has a sufficiently general form of primaeval inhomogeneity). Fragmentation into galaxies can only occur after matter (density ρ_m) and radiation (ρ_r) have decoupled (at $T \sim 0.02$ Rydberg). One has $N_J \sim c^3 t (Gm_p)^{-1}$ when the Universe is still radiation-dominated, with t determined by $\rho_m + \rho_r \sim G^{-1} t^{-3}$, and $\rho_r \sim (kT/hc)^3 (kT/c^2)$.

Consequently, N_J can be expressed in terms of fundamental constants by adopting a temperature equal in order of magnitude to a Rydberg, yielding $N_J \sim S^{1/2} \alpha_g^{-3/2} \alpha^{-3} (m_p/m_e)^{3/2}$, or $\sim 10^{16} (S/10^8) M_\odot$. Here the mean entropy per baryon $S \sim 10^8$ is equal to the number of cosmic blackbody radiation photons per hydrogen nucleus and is approximately constant during the course of the nearly isentropic cosmological expansion. We thus account for the order of magnitude of S by noting that N_J must at least be comparable in mass to a supercluster of galaxies. This suggests that the entropy of the Universe is causally

related to its large-scale structure.

Strictly speaking, this argument provides only a lower limit on S for an assumed value of α_g ; inhomogeneities on scales larger than superclusters could also exist. An upper limit on S is found by requiring that the dynamics of the Universe be matter-dominated (thereby enabling gravitational instability to proceed, to allow formation of galaxies and stars) when the age of the Universe is first comparable with the lifetime of a star. To evaluate this condition, one can set $t \sim \alpha_g^{-1} (h/m_p c^2)$ in the above expression for ρ_r , yielding $kT/m_p c^2 \sim \alpha_g^{1/4} (1 + \rho_m/\rho_r)^{-1/4}$. Since $S = (\rho_r/\rho_m) (m_p c^2/kT)$, one concludes that, if $\rho_r < \rho_m$, one must S have $\lesssim \alpha_g^{-1/4}$. Thus, knowledge of α_g is essential for determining S .

A lower bound on α_g can be obtained independently of S by noting that for galaxies to exist, larger primordial inhomogeneities must be present, whence $N_J \gg N_{\text{gal}}$. This condition yields $\alpha_g \gg \alpha^{16} (m_e/m_p)^2 S^{-1}$, and using the upper bound on S just given, one concludes that $S^{-4} \gtrsim \alpha_g \gg \alpha^{64/3} (m_e/m_p)^{8/3} \approx 10^{-54}$. Therefore the finite entropy of the Universe strongly constrains α_g , and is intimately related to the weakness of the gravitational interaction.

The formation of galaxies and stars provides an insight into the magnitude of α_g . Moreover, it seems plausible that the existence of large-scale structure can only occur in a universe with sufficiently great entropy per baryon. Thus one returns (perhaps inevitably) to a modified form of the anthropic hypothesis: gravitational instability and fragmentation must lead from giant clusters to galaxies to stars, and ultimately to planets and an environment suitable for the development of life. This unbroken chain is essential in any cognisable universe, and may therefore provide the key to understanding the significance of the fundamental dimensionless numbers of astrophysics and cosmology.

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A method for determining the cosmological deceleration parameter q_0

THE availability of deep plates from the UK 48-inch Schmidt combined with the ability to measure the apparent magnitude of large numbers of images using the Royal Observatory Edinburgh COSMOS machine has made possible a new experiment to determine the cosmological deceleration constant q_0 . We have constructed a number-magnitude plot for a large sample of images and compared this with a number of simulated curves representing different values of q_0 .

The coordinates of the plate centre were: right ascension 2 h 41 min 00 s, declination $-30^\circ 00'$ which is a field near the South Galactic Pole. The plate was exposed for 60 min on December 8, 1974, using a combination of unsensitised Eastman Kodak 098 emulsion and a Schott RG630 filter and was measured on the Royal Observatory Edinburgh COSMOS machine using the coarse measurement mode (CM) (ref. 1). The total area of the plate measured was 12.5×13.5 cm and the plate scale was 67 arc s min^{-1} .

The CM mode measures the extents, centroids, blackness and area of the images above the plate background. COSMOS currently scans in units of $8 \mu\text{m}$ using a $25\text{-}\mu\text{m}$ spot full width

half height. The image area, A , of principal interest in this experiment is digitised in increments of $8 \mu\text{m}^2$.

A is a measure of the area of an image above the computed sky background. The relationship between $\log A$ and apparent magnitude m has been investigated². These results, from a number of Schmidt plates, show the relationship to be linear, of the form

$$m = b \log A + \text{const} \quad (1)$$

throughout the range $1.0 \leq \log A < 2.2$. The parameter b varies slightly from plate to plate, as may be expected, and in the present case, $b \approx 3.5$.

A total of 41,225 images was detected above the plate threshold but only images of 10 increments or greater were included in the analysis. This corresponds to a minimum image size of 1.77 arc s while the plate was taken in seeing of ~ 1 arc s. The number of images N , in each digitised area bin A was plotted logarithmically in Fig. 1.

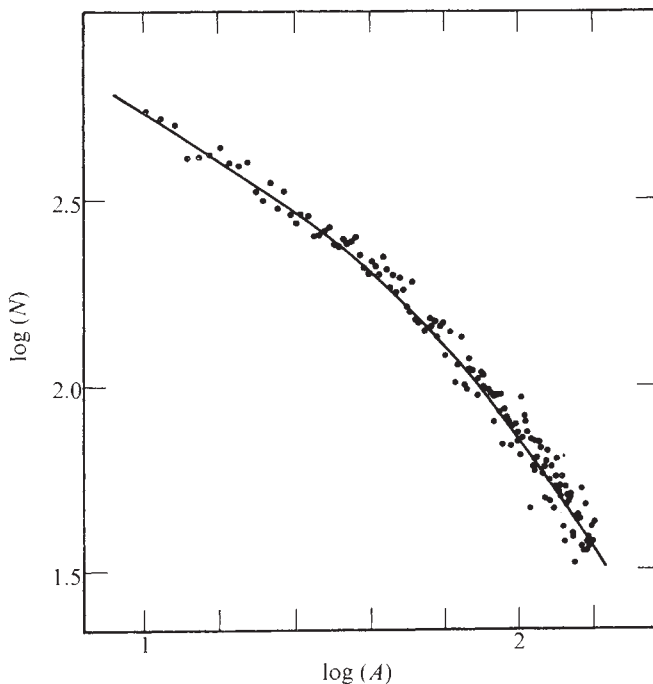


Fig. 1 Log N -log A relationship showing best fit curve to observed data. Dots represent number of images in each area bin.

A preliminary examination of the log N -log A plot in Fig. 1 shows a noticeable change in slope in the region of $\log A \approx 1.5$. To determine the cause of this a simulated number-magnitude plot was constructed on the basis of the absolute fluxes for galaxies given in ref. 3. The procedure adopted was as follows. k -corrections were evaluated by computer for each galaxy type with the effective wavelength bandpass given by the filter-photographic plate combination described above. The k -correction is applied to the apparent magnitude to compensate for the Doppler shift of the spectrum across the filter combination and is a function of the galaxy type. It is defined by

$$k(z) = 2.5 \log(1+z) + 2.5 \log \left(\frac{\int_0^\infty F(\lambda) S(\lambda) d\lambda}{\int_0^\infty F\left(\frac{\lambda}{1+z}\right) S(\lambda) d\lambda} \right) \quad (2)$$

where F is the intrinsic flux distribution of the galaxy as a function of wavelength and S is the effective detector response.

Mattig⁴ has shown that a galaxy of absolute magnitude M at redshift z will have an apparent magnitude m , which, with

the addition of the k -correction, is given by

$$m = M + 5 \log D(z) + k(z) + \text{const} \quad (3)$$

$$\text{where } D(z) = \frac{1}{q_0^2} \left\{ q_0 z + (q_0 - 1) (\sqrt{1 + 2q_0 z} - 1) \right\} \quad (4)$$

and q_0 is the deceleration parameter.

The predicted number of galaxies of magnitude m is calculated by computing the volume of space at that redshift. This is given by Mattig as

$$N = \begin{cases} (1-2q_0)^{-3/2} (\phi \sqrt{1+\phi^2} - \text{arc sinh } \phi) & q_0 < \frac{1}{2} \\ 16/3 D^3(1 + D + \sqrt{1+2D})^{-3} & q_0 = \frac{1}{2} \\ (2q_0-1)^{-3/2} (\text{arc sin } \phi - \phi \sqrt{1-\phi^2}) & q_0 > \frac{1}{2} \end{cases} \quad (5)$$

$$\text{where } \phi = \frac{D \sqrt{2q_0 - 1}}{q_0(1+D) - (q_0-1) \sqrt{1+2D}}$$

(D defined as in equation (4)).

This calculation is repeated for a range of values of z . The frequency distribution of the absolute magnitudes of galaxies of a particular type is believed to be Gaussian. This luminosity function is given by Pence³ for various galaxy types.

The calculation of the apparent magnitudes was repeated for each galaxy type throughout the range of absolute magnitude, and the total number at each apparent magnitude was then weighted according to the luminosity function. The entire process was repeated for each galaxy type to give the overall distribution of apparent magnitudes.

Thus the predicted relationship between log N and m was obtained for a given q_0 . The simulated curves showed a change in slope over a range of values of q_0 . This is attributable to a marked change in the value of the k -correction due to a broad absorption feature being red-shifted into the filter combination. This suggested the possibility of fitting the simulated data to the observed by varying the value of q_0 in the simulation. To achieve this, the simulated log N - m relationship was evaluated for values of q_0 in the range $0 \leq q_0 \leq 2$ in intervals of 0.1. To determine the minimum r.m.s. difference between the observed and the simulated curves it was necessary to fit for zero points of the simulated log N and m axes since these were not included in the simulation. This process provided a best fit value for q_0 . This value, however, was somewhat dependent on the scaling factor of the magnitude axis which had been included in the simulation (variable b to equation (1)). Therefore, due to the uncertainty in b it was decided to fit this parameter as well. As a crosscheck, we tested the consistency of the method by fitting for a scaling factor in the log N axis.

The fitting was done by a combination of analytic and iterative computer processes for each value of q_0 ; this is equivalent to laying the simulated data on the observed, and aligning and stretching the axes in order to give the minimum r.m.s. deviation between the two curves.

The minimum r.m.s. for each value of q_0 is plotted in Fig. 2. There is a minimum in the region of $q_0 \approx 0.8$. The solid line represents the best fit cubic polynomial to these points, which has its minimum value at $q_0 = 0.81$. The formal s.d. in the fit is $q_0 = \pm 0.04$. A more realistic error is obtained, however, by taking the limits defined by applying the r.m.s. scatter about the best fit to the minimum point which gives $q_0 = 0.8 \pm 0.3$. The simulation corresponding to $q_0 = 0.8$ is plotted as a solid line in Fig. 1.

The magnitude scaling factor obtained from the best fit was $b = 3.45$, which compared favourably with the predicted value 3.5. The log N scaling factor was expected to be 1.0. Evolutionary effects would tend to increase this value, however, implying that objects were either more numerous or brighter in the past. The value obtained was 1.05, which does not differ far enough from unity to indicate a significant evolutionary effect. The smallness of the deviation from unity indicates, however, that

the simulation was an adequate model for the observed distribution.

The possibility of effects other than the k -correction (for example, some data acquisition error) were examined to see if they were capable of causing the shape of the observed distribution.

Extensive tests carried out with COSMOS recently indicate that for image areas greater than 10 increments the systematic errors in the measured areas are small. The smallest images used in the analysis are at least one magnitude above the plate limit and the critical change in slope is approximately $2\frac{1}{2}$ magnitudes above the plate limit. Photographic effects such as reciprocity failure have negligible effect on the image size at this level. A possible source of error is the construction of the model used in the simulation. It is now generally accepted that galaxies were intrinsically brighter in the past⁵. This means that any method of evaluating q_0 which relies on measuring apparent magnitudes should make allowances for evolutionary effects, which tend to increase the measured value of q_0 . This has been discussed in recent papers in which the magnitude-redshift relationship is used to evaluate q_0 ⁶, but as the evolutionary processes involved remain uncertain, it was decided to exclude them from this model. Similarly, effects due to intergalactic absorption, which would tend to decrease the measured value of q_0 , but which also are uncertain, have also been ignored. However, the proximity of the log N scaling factor to unity gives weight to the argument that the sum of these effects is small.

One possible source of error in the model was the absence of stars in the simulation although some stars will appear in the observed data. It may be deduced from recent observations of the stellar luminosity function near the South Galactic Pole that the proportion of stars to galaxies in the observed data is probably small^{5,6}. Nevertheless, an attempt was made to incorporate stars into the simulated model, but the effects were small. The value of q_0 could be reduced by up to ~ 0.4 , but at present there is a large uncertainty in the stellar luminosity function at these magnitudes. Therefore for the final result the stars were excluded. It should be pointed out, however, that a proportion of the images in the observed data were of 'stellar' appearance. It is probable that these objects are predominantly distant galaxies where only the compact central region is observed, as would be expected from known galaxy profiles.

Again, the derived scaling factor of the log N axis indicates that there was no appreciable contamination of the data by stars.

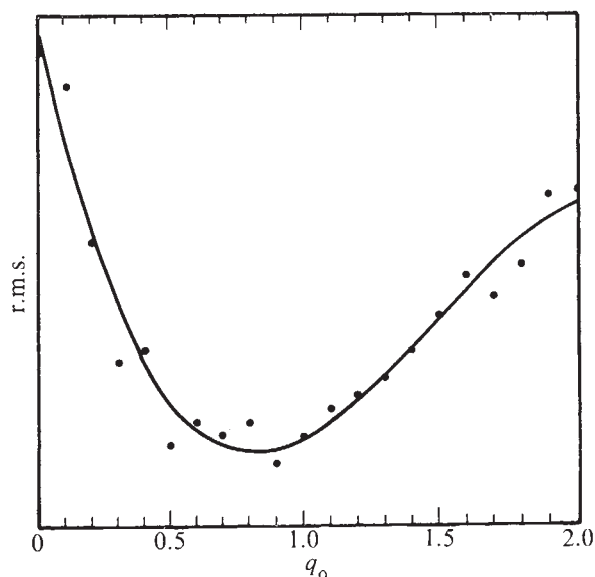


Fig. 2 r.m.s. deviation of best fit curve for each value of q_0 from the observed data in Fig. 1.

The result of this experiment gives a formal value

$$q_0 = 0.8 \pm 0.3$$

It should be possible to estimate the size of some of the possible systematic errors by further analysis of independent samples. We intend to repeat the experiment with deeper plates and a calibration system that is currently under development.

This experiment has the advantage that the observed data may be assumed to contain a complete sample of intrinsic galaxy types throughout the redshift range considered. This means the estimated value of q_0 is independent of the Hubble constant and the absolute magnitude of the galaxies.

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Sunspot cycles and solar activity forecasting

I REPORT here that there is a linear relation between the relative intensity of solar cycle activity, as measured by the ratio (μ) of maximum annual mean sunspot number of two consecutive cycles, and the relative skewness of the first cycle. This relationship facilitates the forecasting of the intensity of the next cycle towards the end of a current cycle. The solar cycle just starting is likely to be more active, with an expected maximum annual average sunspot number of 195, compared with 106 for the cycle just ended.

The solar cycles as represented by the mean annual sunspot number for the period of observations beginning in the year 1756 reveal no obvious pattern in the variations of solar cycle activity as measured by the maximum annual sunspot number. The cycles are all asymmetrical. The degree of asymmetry of the cycles may be represented by the statistical parameter of relative skewness (β), where

$$\beta = \frac{m_3}{\sqrt{(m_2^3)}} \quad (1)$$

and m_3 is the third moment about mean, or $(\sum f_i x_i^3)/\sum f_i$, with f_i the frequency and x the deviation about the mean; $m_2 = S^2$ is the second moment about mean the S and the standard deviation.

In general, the greater the sunspot activity, the more positive the cycle skewness; this is illustrated in Fig. 1. Cycles with $R_{\max}$ (maximum annual mean sunspot number) less than 100 have $\beta \leq 0.35$ (with one exception), and cycles with higher $R_{\max}$ have $\beta > 0.35$ (with two exceptions). In Fig. 2 the β values of the cycles are plotted against the ratio μ of the $R_{\max}$ of the next

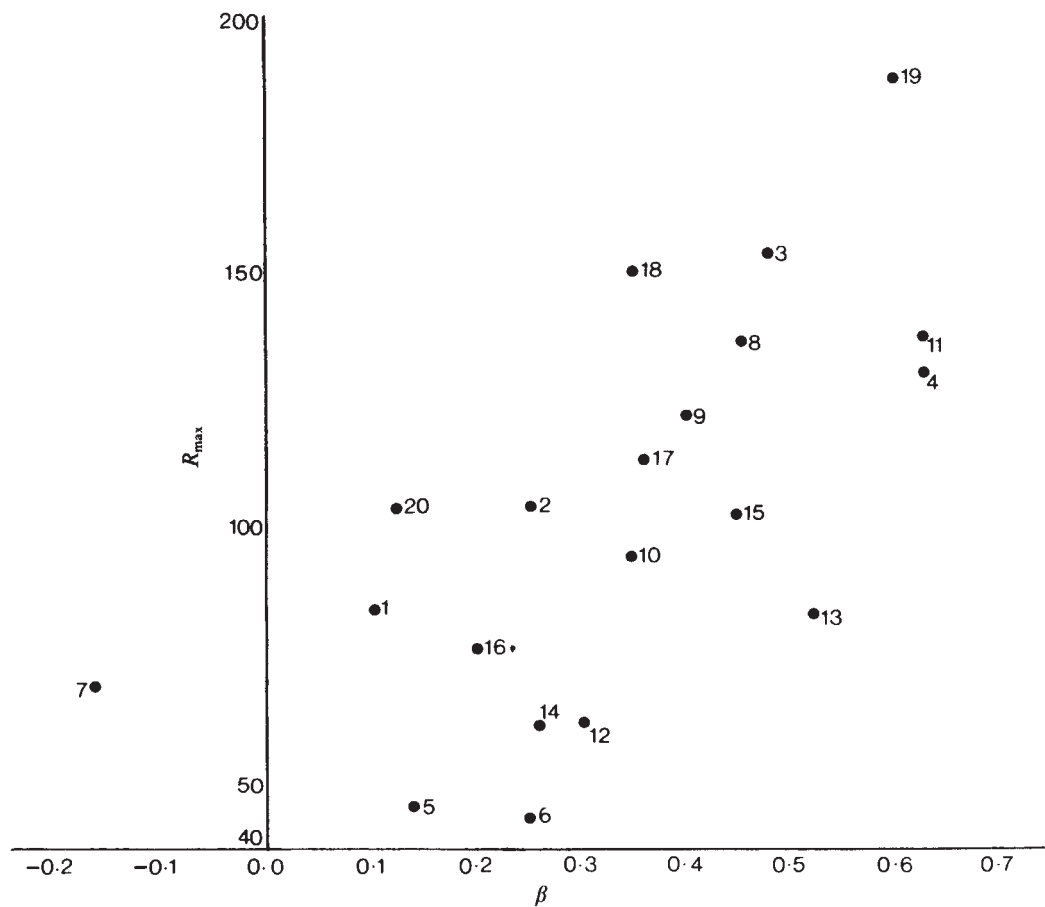


Fig. 1 $R_{\max}$ of cycle (mean annual sunspot no.) at various values of cycle skewness (β). Numbers against points are cycle numbers from cycle 1756–1766.

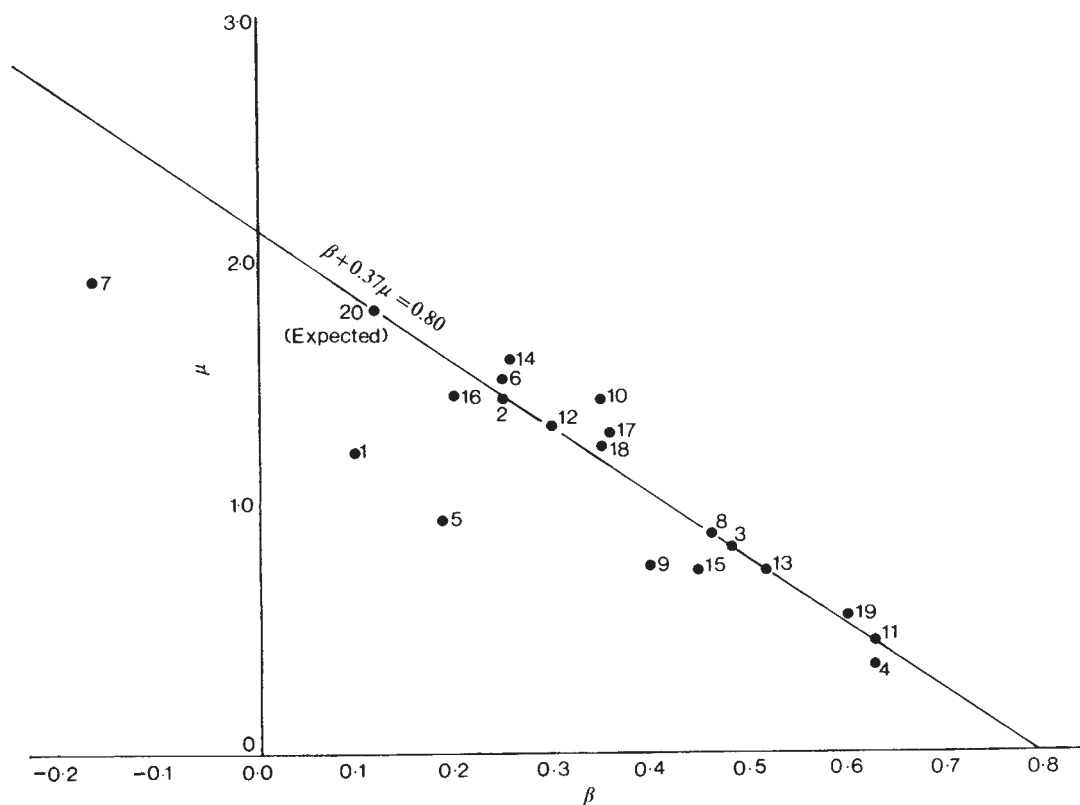


Fig. 2 Ratio of $R_{\max}$ of next cycle to $R_{\max}$ of current cycle (μ) for various values of β . Numbers against points are cycle numbers from cycle 1756–1766.

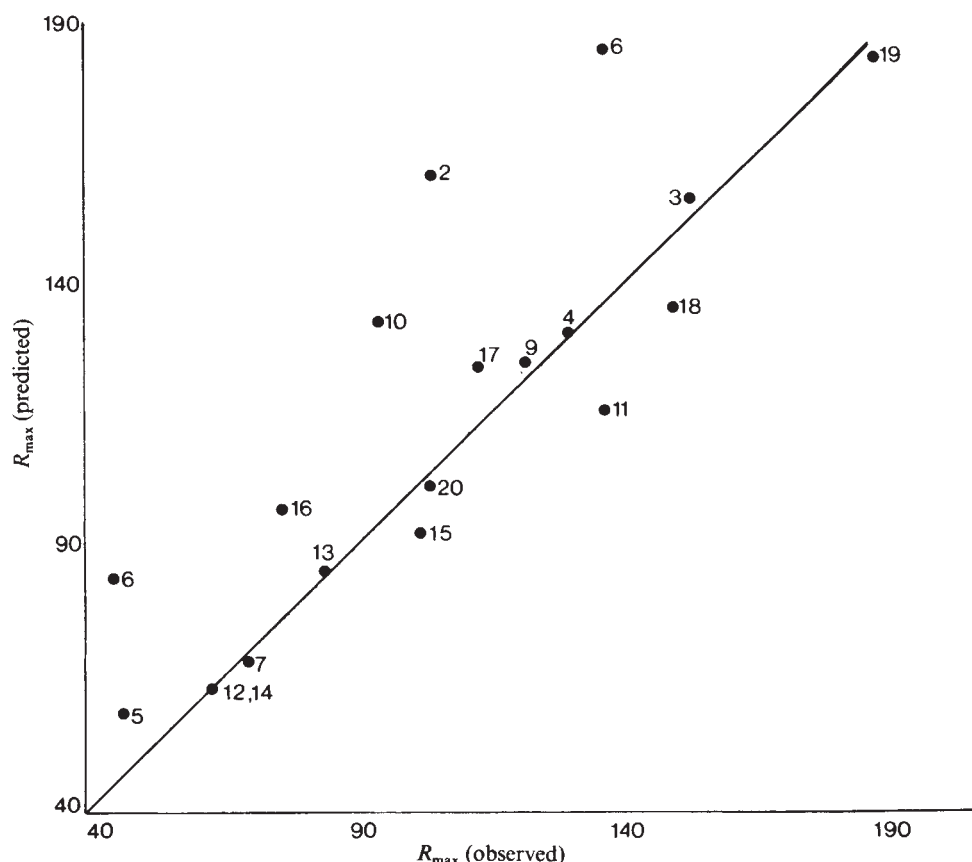


Fig. 3 Predicted and observed values for $R_{\max}$. Numbers against points are cycle numbers from cycle 1756–1766.

cycle and $R_{\max}$ of a current cycle. This plot exhibits linearity and may be represented as

$$\beta + 0.37 \mu = 0.8$$

Figure 3 is a scatter plot of the observed $R_{\max}$ values of the cycles against the $R_{\max}$ values derived from the above linear relation. The scatter about the equidistant line is less than 15% for all but five of the 19 cycles plotted and 50% of the cycles show less than 5% error.

The above empirical relation was established in 1965 (G.R., unpublished thesis), and verified for cycle 20 just ending. Extending it to the coming 21st cycle, it seems that this cycle should be very active. The formula predicts that activity will increase by 1.84 times the activity of the previous (20th) cycle. The expected intensity may thus be close to that of the 19th cycle which was the most active in 200 yr. In 1978, or within 3 yr of the onset of the new cycle, this sunspot high may be observed.

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Theoretical chemistry of superheavy elements E116 and E114

RECENT evidence¹ for the presence of primordial superheavy elements 126, 124, 116 and possibly 114 in microscopic crystalline monazite inclusions in biotite micas raises several interesting questions. Earlier work^{2,3} suggested reasons why E124 and E126, the homologues of U and Pu, respectively, might occur in such an environment, but gives little information

about E116. Better information on both E114 (eka-Pb) and E116 (eka-Po) could enable us to see why they might occur in these minerals and help suggest methods of concentration. At present, *ab initio* relativistic structure calculations for molecules containing superheavy elements are not feasible. We have developed techniques, however, for studying the structure of heavy atoms and ions using the multi-configuration Dirac-Fock (MCDF) relativistic self-consistent field method⁴; this is a considerable advance on the single configuration Dirac-Fock or Dirac-Slater techniques previously available^{2,3}. Applications of the MCDF method to atoms and ions of Ba, Hf (Ref. 4), Bi and U, for example, have yielded good ionisation potentials and energy spectra, mainly due to the way in which relativistic effects, which increase dramatically in size with atomic number²⁻⁴, are handled. These effects make it difficult to predict some chemical properties of superheavy elements by extrapolation from the Periodic Table. We use here, therefore, the results of MCDF calculations of some properties to estimate the heat of formation of simple compounds of E114 and E116 with the simple ionic model⁵ and with Pauling's semi-empirical valence-bond resonance model⁶, to acquire insight into the chemistry of these elements.

The atomic data required are listed in Table 1. Ionisation potentials (IP) were obtained from energy differences of MCDF ground states of the relevant ions, whenever no reliable experimental value was available, adding an energy of 77 kJ mol⁻¹ to correct for correlation effects not allowed for in the calculation^{2,3}. Ionic radii were estimated from the mean radius of the outermost Dirac-Fock orbital using a method to be reported elsewhere; for E114²⁺, this estimate differs by 0.01 Å from the value linearly extrapolated from Sn²⁺ and Pb²⁺. Lattice energies were estimated from the Kapustinskii equation (ref. 5), taking $\rho = 0.345$ in the Born-Mayer repulsion formula. The overall uncertainty in the lattice energy is too small to affect our conclusions; we shall report the details of our investigations elsewhere. Heats of sublimation, if not available, were estimated

by extrapolating down the relevant group of the Periodic Table; their contribution is dominated by the ionisation potentials, and extrapolation errors are unimportant for our purposes.

The data for the covalent model⁶ were estimated in a similar way. The MCDF program was used to compute the energy, E_v , needed to excite a superheavy atom to a valence state which can form strong bonds. This is an important contribution for E116 and E114, whose ground states are almost pure jj coupled; such states contain a substantial anti-bonding component⁹. Homonuclear bond strengths, $D(X-X)$, were estimated following Phillips and Williams⁷, and heteronuclear bond strengths, $D(X-Y)$, from Pauling's formula⁸. The unknown electronegativities, χ_v , were found from Mulliken's formula⁸,

fluoride and sulphate are predicted stable, contradicting a conjecture⁹, based on cruder estimates ($E_v(\text{Pb}) = 192 \text{ kJ mol}^{-1}$, $E_v(\text{E114}) = 598 \text{ kJ mol}^{-1}$), that E114 might behave as a relatively inert gas.

The differences between the results of the two models for E116 compounds are so large, and so different from those for polonium, that we conclude that divalent halides of E116 will be stable and essentially ionic, and that the salts of Table 3 should be capable of preparation. Examination of contributions to the Born-Haber cycle shows that this is due to the weak binding of $7p_{3/2}$ electrons ($\epsilon = 632 \text{ kJ mol}^{-1}$); the value of IP_2 , 1937 kJ mol^{-1} , lies between those for Ca (1736 kJ mol^{-1}) and Mg (2188 kJ mol^{-1}). Since E116^{2+} has a larger ionic radius

Table 1 Atomic properties

Atom	r_{+2} (Å)	r_{+4} (Å)	ΔH_s	IP_2	IP_4	E_v^*	χ_v	$D(X-X)$
Te	1.28	0.94	197	2,665	8,966	75	2.1	138
Po	1.32	0.97	142	2,364	8,640	197	1.8	105
E116	1.35	1.01	109	1,937	8,711	577	2.0	79
Pb	1.21	0.84	197	2,167	9,330	134	1.8	109
E114	1.29	0.91	126†	2,531	10,247	427	1.6	96

Heats of sublimation (ΔH_s), ionisation potentials (IP_2/IP_4 for removal of two/four electrons, respectively), valence excitation energies (E_v) and homonuclear bond strengths ($D(X-X)$) are in units of kJ mol^{-1} . Experimental data have been used, when available, for Te, Po and Pb.

* E_v is the promotion energy for divalent p -orbital bonding. The sp^3 tetrahedral quadrivalent state of E114 requires a promotion energy of $1,665 \text{ kJ mol}^{-1}$, making such compounds unlikely.

† This is probably an upper bound. Keller *et al.*³, using a different extrapolation, give 42 kJ mol^{-1} , which would make the predictions of ΔH_f for E114 compounds in Tables 2-4 more stable by about 84 kJ mol^{-1} .

using MCDF estimates of ionisation potentials and electron affinities. Again, we shall report the details elsewhere.

Some care is needed in drawing conclusions from Tables 2-4. We use enthalpy instead of free energy to assess thermodynamic stability; however, the difference is too small to affect our conclusions at room temperatures¹¹. An endothermic compound ($\Delta H_f > 0$) is not necessarily unstable (e.g. H_2Te). An exothermic compound ($\Delta H_f < 0$) is stable against decomposition to the free elements, but can still be unstable with respect to other

(1.35 Å) than Mg^{2+} (0.68 Å), the lattice energies of E116 dihalides will be about 420 kJ mol^{-1} less than those of Mg. The relative instability of covalent E116 compounds is mainly due to the strong binding of $7p_{1/2}$ electrons ($\epsilon = 1414 \text{ kJ mol}^{-1}$) leading to a large valence state promotion energy of 577 kJ mol^{-1} . The E_v and χ_v are larger than expected from extrapolation down Group VIB.

The reliability of our models for E116 can be assessed from the corresponding results for Te and Po. We conclude that

Table 2 Predicted heats of formation of dihalides (kJ mol^{-1})

Atom	Ionic model				Covalent model (gas phase)				
	F ⁻	Cl ⁻	Br ⁻	I ⁻	F	Cl	Br	I	H
Te	-79	297	410	598	-561*	-272	67	163	134
Po	-397	-29	79	264	-699	-46	67	201	213
E116	-837	-473	-335	-155	-163	418	514	623	611
Pb	-628	-238	-121	71	-707	-54	59	192	205
E114	-272	100	218	402	-644	84	209	360	406

* Covalent TeF_2 is unknown, presumably because it is unstable with respect to disproportionation to covalent $\text{TeF}_4 + \text{Te}$.

decomposition or disproportionation reactions. It seems reasonable to predict that a compound is definitely covalent or ionic if the difference in the respective values of ΔH_f is sufficiently large, say 200 kJ mol^{-1} .

Thus, we predict that E114 should be less reactive than Pb, mainly because IP_2 is 313 kJ mol^{-1} larger than one would expect by extrapolating from Sn and Pb. Other contributions to ΔH_f vary smoothly down the group, so that this relativistic atomic effect dominates. The ionic-covalent resonance term, which may here be slightly overestimated, is responsible for the relatively large covalent energy of E114.F_2 . Although the gaseous halides, other than the fluoride and, possibly the chloride (Table 2 footnote) seem to be endothermic, the ionic

tellurium dihalides are all covalent, in agreement with known chemical properties, whilst TeCO_3 and $\text{Te(NO}_3)_2$ are almost certainly unstable with respect to the oxide, and complex tellurium sulphates are known¹⁰. Similarly, the emergence of cationic properties for polonium is correctly predicted.

The ionic radius of E116^{4+} (1.01 Å) and the energy required to ionise the two $7p_{1/2}$ electrons (6774 kJ mol^{-1}) are larger than those of Po^{4+} (0.97 Å and 6276 kJ mol^{-1}), making its quadrivalent ionic compounds less stable than those of polonium. Whilst polonium forms volatile PoCl_4 , PoBr_4 and PoI_4 , which are presumably covalent in the gas phase, the E116 analogues seem unlikely to be stable. The covalent dihalides seem to be strongly endothermic, and the energy released by forming two more bonds would not be sufficient for stability when the promotion energy of a p^3d valence state is taken into account. Hexavalent E116 compounds seem unlikely to be stable because the $7s-7p_{3/2}$ energy gap (2063 kJ mol^{-1}) is much larger than the corresponding $6s-6p_{3/2}$ gap (1330 kJ mol^{-1}) in polonium.

We conclude that the chemistry of E116 should be mainly cationic; although it is in Group VIB, we expect it to form divalent ionic compounds with an ease approaching that of beryllium or magnesium, and quadrivalent compounds with the most electronegative atoms. The element might reside in monazite as

Table 3 Predicted heats of formation of divalent inorganic salts using the ionic model (kJ mol^{-1})

Atom	SO_4^{2-}	CO_3^{2-}	NO_3^-	PO_4^{3-}
Te	-226	-21	180	-347
Po	-552	-343	-151	-1,318
E116	-971	-782	-569	-2,641
Pb	-761	-569	-356	-1,983
E114	-418	-213	-17	-929

Table 4 Predicted heats of formation of quadrivalent compounds using the ionic model (kJ mol⁻¹)

Atom	F ⁻	Cl ⁻	Br ⁻	I ⁻	SO ₄ ²⁻	CO ₃ ²⁻	NO ₃ ⁻	PO ₄ ³⁻ †
Te	-900	552	966	1,619	-92	-193	397	233
Po*	-1,180	326	653	1,243	-418	-485	88	-682
E116	-908	377	774	1,351	-297	-339	213	-285
Pb	-866	678	1,109	1,732	377	-113	531	594
E114	218	1,695	2,113	2,715	1,042	937	1,540	3,653

*The sulphate, carbonate and phosphate of polonium are all known experimentally.

†Neglects the unknown heat of formation of the gaseous phosphate ion. Attempts to estimate this quantity from known phosphates gave numbers ranging from +8 to -364 kJ mol⁻¹.

a divalent phosphate or as a quadrivalent phosphate or mixed oxide, the sizes of E116⁴⁺ and Ce³⁺ being very similar. The presence of small quantities of Ca²⁺ and Mg²⁺ ions in the mineral has been noted¹⁰.

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been used only as a standard of relative spectral distribution, since the circulating electron current cannot be measured with sufficient accuracy to establish absolute levels. These are therefore set by comparison at about 350 nm with existing visible/near-ultraviolet standards based on conventional blackbody radiators. Work at Daresbury has extended this scale into the vacuum ultraviolet, currently to a limit set at about 165 nm by the availability of suitable transfer standard sources. These are normally deuterium discharge lamps, producing continuum radiation from 165 to 370 nm, and are specially modified and selected versions of commercially available lamps, with ageing rates of less than 0.05% per h at all wavelengths. The accuracy now attainable in the calibration of these sources is estimated as $\pm 2.7\%$ at 200 nm and $\pm 3.6\%$ at 165 nm.

To effect the intercomparison, four deuterium lamps were calibrated using synchrotron radiation and their spectral radiances then determined by the argon arc method at the three resonance lines, 165.7 nm (Cl), 174.3 nm (Ni) and 193.1 nm (Cl). The measurements were made with a very high resolution concave grating monochromator to investigate adequately the saturation of the peak of the blackbody resonance lines. The aperture ratio of the optical system ($f/260$) was determined by the aper-

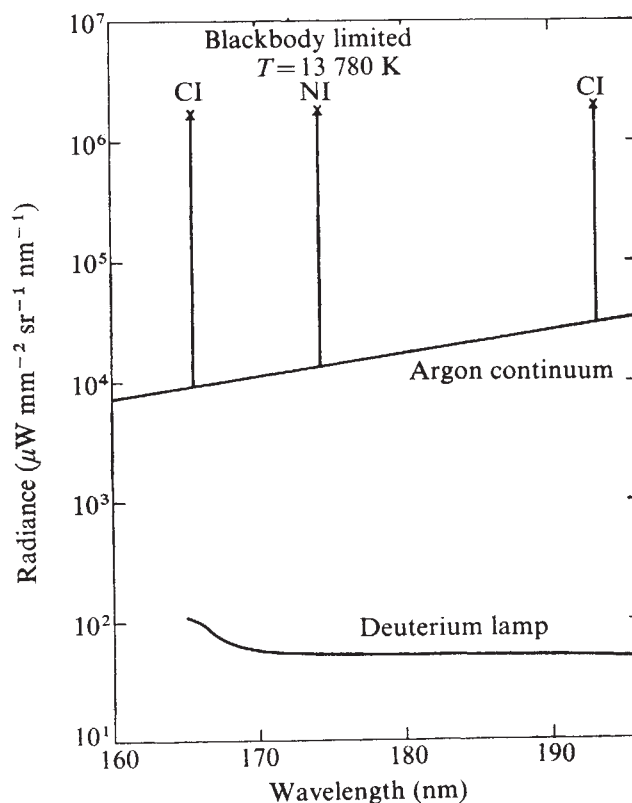
Vacuum ultraviolet radiation scales

WE report here a comparison of two independent techniques (high-temperature argon arc and synchrotron radiation) for the determination of vacuum ultraviolet spectral radiances. Basic agreement between the methods has previously been demonstrated at 165.7 nm by Stuck and Wende¹, but we have refined the techniques to produce significant improvements in accuracy and have extended the comparison to other wavelengths.

The addition of trace gases to a high-temperature wall stabilised argon arc results in the production of optically thick resonance lines (for example from C, Kr, N, O) at wavelengths predominantly in the vacuum ultraviolet². The determination of the blackbody limited spectral radiances at the centres of these lines requires only a measurement of plasma temperature. Recent research at NPL (R.C.P., in preparation) has produced increased accuracy in such temperature determinations (± 55 K at 13,800 K) as a result of the application of various plasma diagnostic techniques. There have also been refinements in techniques for producing saturation of the resonance line radiation with minimal perturbation to the plasma, and in correcting for the effects of the residual perturbation. As a result, resonance line radiances can be established with a marked improvement in accuracy, for example $\pm 3.2\%$ at 165.7 nm and $\pm 2.6\%$ at 193.1 nm.

The method based on the predictable spectral emission properties of synchrotron radiation^{3,4} is well established. In the past few years we have used several different experimental techniques, with both the Glasgow University and the SRC Daresbury synchrotrons, to set up an accurate ultraviolet spectral emission scale (P.J.K., in preparation), initially down to 200 nm. The synchrotron radiation has

Fig. 1 Radiances of resonance lines, argon continuum and a typical deuterium lamp.



tures in the differential pumping system and by the need to restrict the field of view to regions of adequate temperature uniformity. The arc was 55 mm long and run at 60 A in argon at a pressure of 1.75×10^5 Pa, producing an axial temperature of 13,780 K. The radiance levels of the line peaks, the argon continuum and a typical deuterium lamp are shown in Fig. 1. Because of the large dynamic range, the intercomparison was made by first measuring the ratio of resonance line peak to argon continuum and then, with an increased exit slit width, the ratio of argon continuum to deuterium lamp radiance.

A major experimental problem arose because the spectral distribution of the deuterium lamp radiation varied significantly from the centre to the edge of the 1-mm diameter emitting area. Synchrotron measurements were based on a 0.9-mm diameter sample, arc measurements on a 0.2×0.03 mm central spot, and detailed investigation revealed correction factors that were large and very wavelength dependent, especially at shorter wavelength ($\sim 7\%$ at 165.7 nm). Another difficulty at 165.7 nm was the onset of the complex deuterium molecular line emission spectrum superimposed on the continuum. Different spectral bandwidths in the synchrotron and arc calibration systems required computer-based convolutions of a high resolution deuterium spectrum to reduce radiance measurements to the same bandwidth conditions. Since the calculated correction factors were very sensitive to wavelength, a further measurement of the deuterium lamp emission was made at 167 nm, away from the line emission, by comparison with the argon arc continuum. This continuum level was in turn obtained by comparison with the 165.7-nm line, the change in instrumental response over this wavelength interval being small enough to be measured with adequate accuracy.

The results of measurements on the four lamps are shown in Table 1. The result at 167 nm is based on the

Volatilisation of methylmercuric chloride by hydrogen sulphide

THE interconversion of mercury compounds in the environment has attracted considerable interest, especially the synthesis and breakdown of the highly toxic methylmercury. The alkylmercurial can be formed and degraded by microorganisms in lakes, rivers, soil and the mammalian intestinal tract¹⁻⁶, although the ecological significance of these processes is difficult to assess. We describe here a further reaction which may be important with respect to the fate of mercury in the biosphere—the interaction of methylmercuric chloride (CH_3HgCl) with hydrogen sulphide. Hydrogen sulphide is produced by many bacteria in anaerobic conditions such as are found in the sediments of lakes and rivers, and it has been shown that trimethyllead salts can be converted to the volatile tetramethyllead by reaction with H_2S (ref. 7).

Methylmercuric chloride labelled with ^{203}Hg (Radiochemicals Centre, Amersham), or ^{14}C (New England Nuclear) was purified by extracting it from aqueous solution into benzene, evaporating the organic solvent and redissolving the residue in distilled water.

When solutions of the purified CH_3HgCl were incubated in the presence of H_2S , radioactivity was lost from aqueous solutions within 3–4 d, the rate of loss of radioactivity being similar whether ^{14}C - or ^{203}Hg -labelled CH_3HgCl was used. Analysis of the solutions by thin-layer chromatography revealed that all remaining radioactivity was present as CH_3HgCl . When N_2 or air was used instead of H_2S , or when the bottles were incubated under H_2/CO_2 (97%/3%) in an anaerobic jar (GasPak, BBL Ltd), little or no radioactivity was lost (Fig. 1 and Table 1). Other sulphur compounds, such as cysteine and sodium thioglycollate, did not induce volatilisation of CH_3HgCl and although ammonium sulphide did cause some loss of radioactivity, this may be ascribed to the slight dissociation of small

Table 1 Ratios of deuterium lamp radiance determined from arc measurements to that from synchrotron measurements

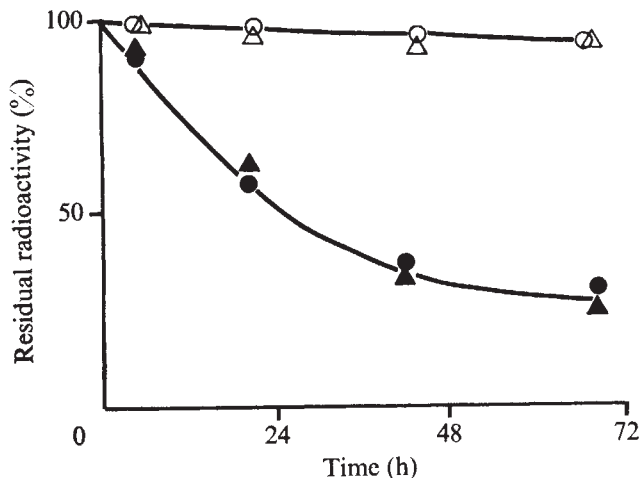
Wave-length (nm)	786	787	Lamp no. 808	810	Mean
165.7	1.009	1.039	1.016	0.993	1.014
167	0.987	1.000	1.004	0.969	0.990
174.3	1.012	0.987	0.998	0.991	0.997
193.1	1.014	0.987	0.995	0.996	0.998

165.7-nm resonance line realisation and so is not totally independent; the difference clearly reflects the problems of the bandwidth and area correction factors. Total intercomparison uncertainties range from 0.9% at 193.1 nm to 2.4% at 165.7 nm and the radiance ratios for all lamps are within the expected limits set by combined intercomparison and basic scale realisation uncertainties. The agreement averaged over the four lamps is extremely good and we conclude that the two methods for establishing vacuum ultraviolet radiances are in agreement to well within their individual scale uncertainties.

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Fig. 1 Volatilisation of CH_3HgCl by H_2S . Solutions of the purified $^{14}\text{CH}_3\text{HgCl}$ ($0.01 \mu\text{Ci } \mu\text{g}^{-1}$, final concentration $2 \mu\text{g ml}^{-1}$) or $\text{CH}_3^{203}\text{HgCl}$ ($0.025 \mu\text{Ci } \mu\text{g}^{-1}$, final concentration $2 \mu\text{g ml}^{-1}$) were incubated at 37°C in screw-cap bottles fitted with rubber septa. H_2S was passed into some of the bottles through hypodermic needles for 2 min before incubation. Samples were taken from the bottles at various times using a syringe and hypodermic needle, so that the atmosphere above the solution was not unduly disturbed. The samples were assayed for residual radioactivity in the solution and for the amount of radioactivity associated with CH_3HgCl by thin-layer chromatography as described previously⁵. $\circ$, $\text{CH}_3^{203}\text{HgCl}$, air; $\bullet$, $\text{CH}_3^{203}\text{HgCl}$, H_2S ; $\triangle$, $^{14}\text{CH}_3\text{HgCl}$, air; $\blacktriangle$, $^{14}\text{CH}_3\text{HgCl}$, H_2S .



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Table 1 Effect of certain gases and sulphur compounds on volatilisation of $\text{CH}_3^{203}\text{HgCl}$ from solution

Compound or gas	Residual radioactivity
Air	98.0 ± 1.2
Nitrogen	97.1 ± 0.9
Carbon dioxide	93.4 ± 1.1
Hydrogen/carbon dioxide (97%/3%)	93.6 ± 2.1
Methane	95.1 ± 1.6
Hydrogen sulphide	30.6 ± 3.0
Sodium sulphide	100.0 ± 0.8
Ammonium sulphide	77.2 ± 1.8
Methionine	97.4 ± 0.5
Cysteine HCl	99.6 ± 1.9
Sodium thioglycollate	98.8 ± 1.6
Sodium thiosulphate	101.6 ± 2.4

Aqueous solutions of $\text{CH}_3^{203}\text{HgCl}$ ($2 \mu\text{g ml}^{-1}$, $0.05 \mu\text{Ci } \mu\text{g}^{-1}$) were incubated for 44 h at 37 °C in the presence of the above substances. The final concentration of the solid compounds was 10 mg ml^{-1} . The results are expressed as percentage of initial radioactivity remaining after 44 h. Figures shown are the means ± s.e. of five results.

amounts of H_2S (Table 1). The inability of the strong reducing agent sodium thioglycollate to volatilise CH_3HgCl suggests that the effect of H_2S on the alkylmercurial is not simply due to the reducing capacity of H_2S . Furthermore, H_2S did not reduce inorganic mercury to metallic mercury (unpublished observations of I.R.R.), which may be compared with the results of De Filippis and Pallaghy⁸, who found that acetylene and ethylene could reduce mercuric salts to mercury metal. The volatilisation of CH_3HgCl cannot be ascribed to the acidic character of H_2S , even though the pH of the CH_3HgCl solution had decreased from 6.5 to 4.0 by the end of the incubation. Experiments in which CH_3HgCl was incubated in air or H_2S in the presence of buffers of pH 2.2–8.5, demonstrated that the pH of the solution had little influence over the rate of volatilisation by H_2S and, furthermore, in the absence of H_2S , virtually no radioactivity was lost at any of the pH values.

The rate of volatilisation increased linearly with concentration in the presence of H_2S , so that the percentage of CH_3HgCl volatilised was constant at all concentrations up to $50 \mu\text{g ml}^{-1}$, the highest concentration tested. The rate of volatilisation was temperature dependent—at 37 °C about 90% of added alkylmercurial was lost from solution, whereas at 5 °C only 50% was lost.

We attempted to trap and identify the volatile product of the reaction between H_2S and $\text{CH}_3^{203}\text{HgCl}$ or $^{14}\text{CH}_3\text{HgCl}$ using a solution of sodium carbonate and disodium phosphate⁹ (Table 2). Nearly all the evolved radioactivity, whether ^{203}Hg or ^{14}C , was absorbed by the carbonate-phosphate solution. In the absence of H_2S , little radioactivity was lost from solution and virtually none was found in the trap. In experiments described in Table 2 there was considerable variation in the amount of mercury

Table 2 Absorption of volatile mercury compound by carbonate-phosphate solution

Conditions	Radioactivity	% Radioactivity lost from flask	% Absorbed by CO_3/PO_4
H_2S	$\text{CH}_3^{203}\text{HgCl}$	23.3	22.9
	$^{14}\text{CH}_3\text{HgCl}$	16.3	14.4
Air	$\text{CH}_3^{203}\text{HgCl}$	1.3	0.0
	$^{14}\text{CH}_3\text{HgCl}$	0.05	0.06

A solution of $\text{CH}_3^{203}\text{HgCl}$ ($2 \mu\text{g ml}^{-1}$, $0.025 \mu\text{Ci } \mu\text{g}^{-1}$) or $^{14}\text{CH}_3\text{HgCl}$ ($2 \mu\text{g ml}^{-1}$, $0.01 \mu\text{Ci } \mu\text{g}^{-1}$) in a conical flask was placed in a desiccator which contained in its base a solution of sodium carbonate (10 g l^{-1}) and disodium phosphate (5 g l^{-1}). In two experiments the desiccator was flushed with H_2S for 2 min. After incubation for 3 d at 37 °C, samples were removed from the flask and from the absorber solution to assess the amount of radioactivity volatilised and absorbed.

volatilised, probably due to differences in initial H_2S concentration, or slight leakages from the desiccator seals. Consequently the differences between percentage loss of ^{203}Hg - or ^{14}C -labelled methylmercury have no significance, for the experiments were performed on separate occasions.

The identity of the volatile product has not been fully elucidated. It does not seem to be metallic mercury because: (1) both ^{14}C - and ^{203}Hg -labelled volatile products were absorbed to the same extent by the carbonate-phosphate solution, which suggests that the C–Hg bond remains intact, and (2) metallic mercury vapour is not absorbed by the carbonate-phosphate solution which traps only organic mercury compounds⁹.

When the radioactive contents of the carbonate-phosphate absorption solution were chromatographed on thin-layer plates of silica gel G-25 (Camlab, Cambridge), using two solvent systems, chloroform–*n*-hexane (9:1) (ref. 10), and *n*-hexane–acetone (7:3) (ref. 11), the radioactivity remained at the origin. But when the contents of the trap were extracted into toluene, all the radioactivity passed into the toluene phase and the radioactivity then migrated on the thin-layer plates at the same rate as a methylmercuric chloride standard, in the two solvent systems. Thin-layer chromatography of a sample of the radioactivity remaining in the flask revealed that it was CH_3HgCl and that no inorganic mercury was present. Thus, it seems likely that CH_3HgCl and H_2S react to produce a volatile sulphur derivative of methylmercury which, although stable in alkaline solutions, reverts to methylmercury on extraction into a polar solvent.

Fagerström and Jernelöv¹² have suggested that the presence of H_2S in sediments of lakes reduces the rate of methylation of mercury, due to the formation of mercuric sulphide, which is methylated much more slowly than mercuric chloride. The results reported here suggest that H_2S plays a further role in the turnover of mercury in an aquatic environment, that is, that any methylmercury discharged into a lake, or synthesised there by microbial action, may react with H_2S to form a volatile product which is released from the lake, thus making less methylmercury available for accumulation by fish and shellfish.

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Mixing in upper layer of a lake during heating cycle

LITTLE is yet known about the structure of the upper 'mixing' layer of the ocean below the zone of direct surface wave influence. We report some measurements of the temperature field in the mixing layer of a freshwater lake during the heating cycle which demonstrate some of the processes which may be important. The temperature field is found to be isotropic at scales less than about 60 cm, but the effect of stratification becomes important at larger

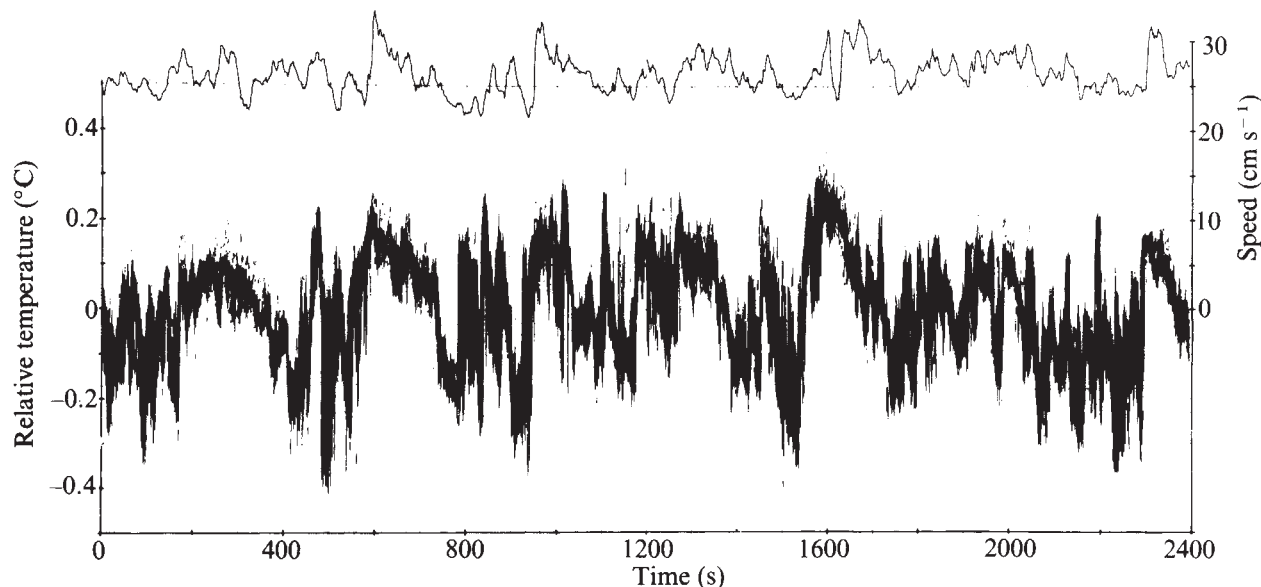


Fig. 1 Temperatures relative to their means and current recorded by the array of thermistors plotted against time. The record starts at 119 GMT, July 14, 1976, when the centre of the array was at 6-m depth.

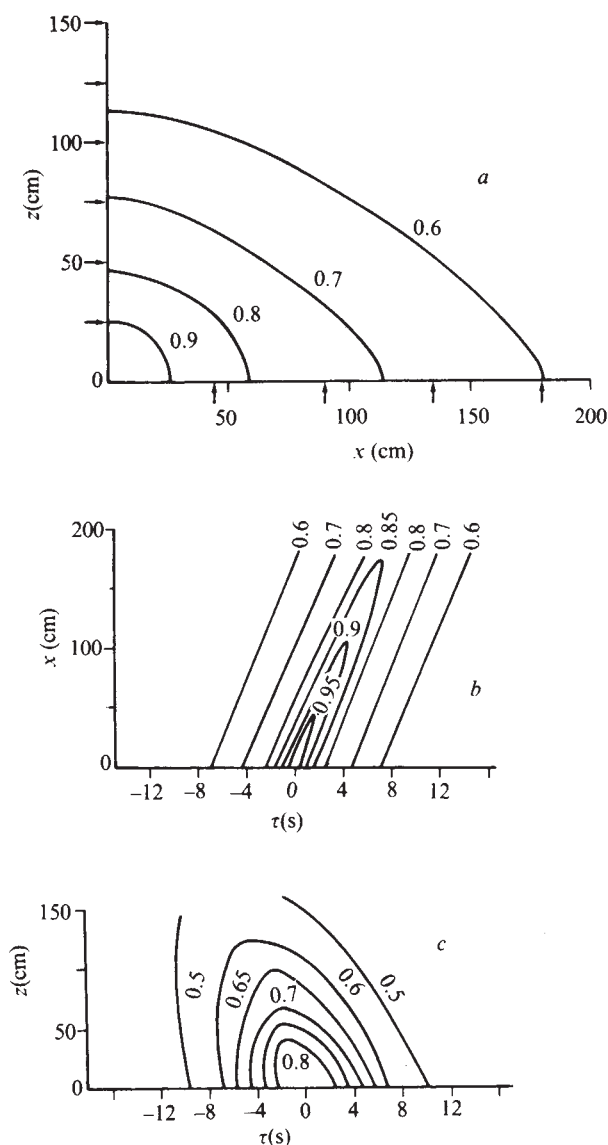


Fig. 2 Sections of the correlation function $C(x, y, z, \tau)$. a, Section $y = \tau = 0$. b, Section $y = z = 0$. c, Section $x = y = 0$.

scales, reducing the vertical correlations in comparison with the horizontal. The spatial evolution of structures is dominated by the shear, as appropriate in the low Richardson number region. Examination of the stability of the mean velocity and density profiles shows that Kelvin-Helmholtz instability may be expected, leading to billows with wavelengths of about 60 m, and corresponding to fluctuation scales observed in the temperature records. Similar fluctuations have been seen in the nocturnal atmospheric boundary layer.

The measurements were made with a thermistor array in Loch Ness, 250 m from the north-east shore near Achnahannet, where the water depth is 170 m. The fetch at this site is over 20 km when south-west winds are blowing along the axis of the loch. The array consists of seven thermistors at 25-cm separation on a vertical rod, five at 45-cm separation in the direction of flow (orientation being maintained by a vane on the supporting tube) and three at 45- and 90-cm separation in the horizontal across the direction of flow. The sampling rate is 1 Hz and resolution 0.001°C . The loch is freshwater, so density can be inferred from the temperature measurements. The array also carries a Savonius rotor current meter, a small vane and compasses to determine the flow direction.

Figure 1 shows the relative temperature values against time. The mean air temperature was about 1.8°C in excess of the surface water temperature, and the wind speed was 10.6 m s^{-1} from the south-west. The dominant surface wavelength was estimated as 10 m, and whitecapping was frequent. There are fluctuations in temperature with a period of about 6 min and with a vertical scale which exceeds the array dimensions (the coherence between thermistors being high), as well as less coherent rapid fluctuations. The current speed is also shown in the figure and was directed in the wind direction with a variation of less than 20° .

We define the four-dimensional correlation function $C(x, y, z, \tau)$, the cross correlation between thermistors at relative position (x, y, z) at time lag τ by

$$C(x, y, z, \tau) = \frac{\overline{T(x, y, z, \tau + t) \cdot T(0, 0, 0, t)}}{(\overline{T^2(x, y, z, t)} \cdot \overline{T^2(0, 0, 0, t)})^{1/2}}$$

$T(x, y, z, t)$ is the variation of the temperature from its mean value, and is recorded at time t by a thermistor at a position (x, y, z) , where x is directed north-east along the loch axis in the wind direction, y is across and z is vertically upwards. The overbars imply means taken with respect to time. Figure 2 shows sections of the constant C surfaces. For thermistor spacing $< 60\text{ cm}$ the $y = \tau = 0$ correlations in directions x and z are large and almost equal, but for larger scales the correlation in the vertical decreases

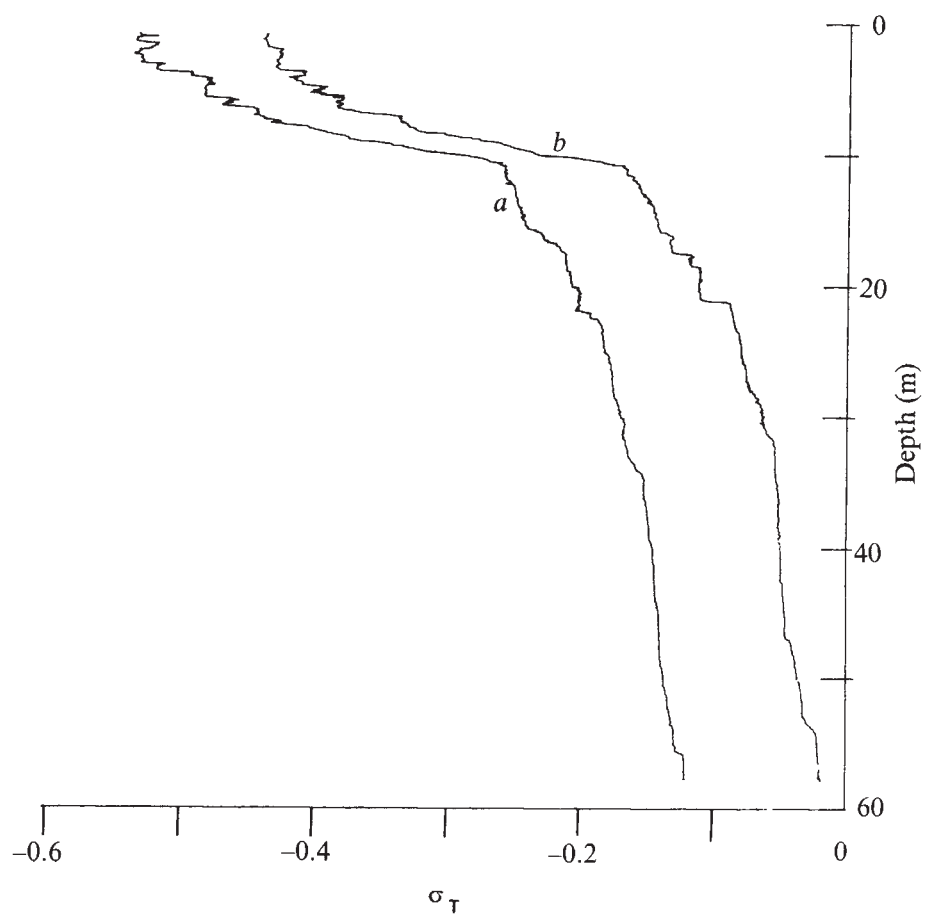
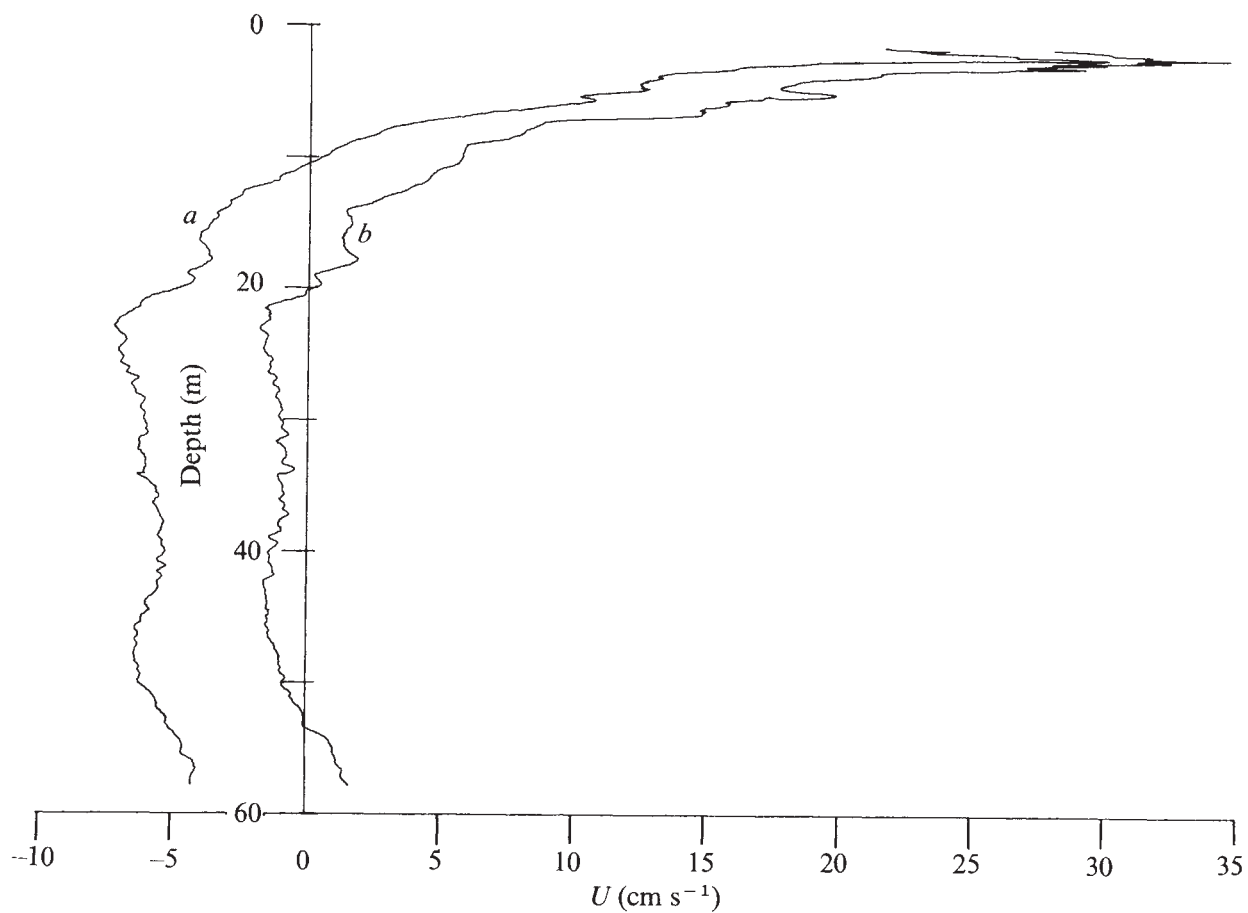


Fig. 3 Two vertical profiles of horizontal current U in direction $036 \pm T$ along the axis of Loch Ness, and of σ_T , made by a profiling current meter¹ immediately before the temperature record. The scales apply to profile a . Profile b has been displaced 5 cm s^{-1} or 0.1 sigma-T units to the right.

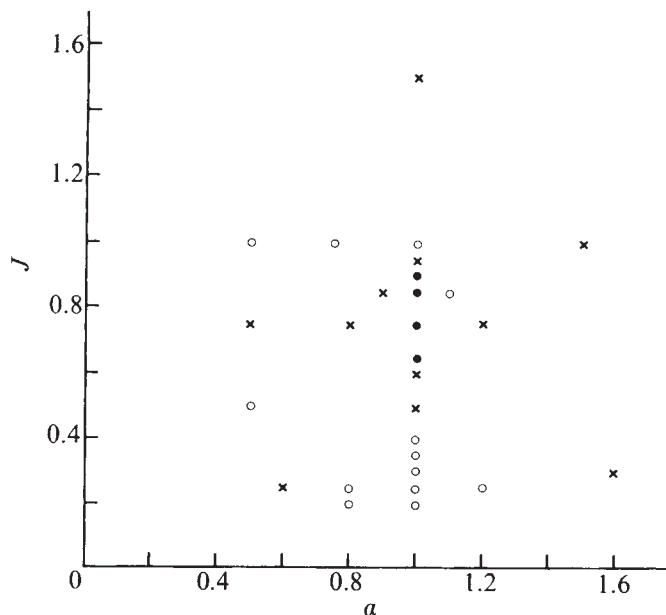


Fig. 4 The wave number, α , and Richardson number, J , plane, showing positions at which stable and unstable waves are found. The wavelength is $20\pi/\alpha$ (m) and the $J=1$ corresponds to the observed mean profile. $\times$, stable waves; O , waves which are unstable but have e-folding growth time exceeding 1 h; $\bullet$, unstable waves with shorter growth times.

more rapidly than in the horizontal, the ratio of horizontal to vertical scales at which $C = 0.6$ is reached being 1.59. The tilt of the constant C contours in the x - τ surface indicates the downstream advection of coherent structures through the array at a velocity which is, within the accuracy of the measurement, the same as the flow velocity. If the contours were all parallel, the correlation pattern would not change in time as it is advected past the array and the temperature field would be 'frozen' (the Taylor hypothesis). Figure 2b indicates that C decreases to 0.85 at $\tau = 7$ s as the pattern is advected and so, by linear extrapolation, $C = 0.6$ at about 19 s, and this is the evolution time scale of the temperature field. Figure 2c shows that the C contours in the z plane are also slightly skewed, indicating a downward propagation of coherent structures. No evidence of horizontal anisotropy was found in the analysis of the y correlations.

Figure 3 shows two profiles of current U and σ_t . The layers above 10 m are subject to a strong shear and contain frequent inversions in which the density decreases with depth. These have a r.m.s. scale of 76 cm, corresponding approximately to the scale at which the vertical coherence falls to about 0.7 and the anisotropy is first found between the vertical and horizontal structures. The Brunt-Väisälä period in this upper layer is about 600 s, so that the evolution scale of the thermal structures is not consistent with their

being internal waves, which should have longer periods. The time scale of vertical shear, $(du/dz)^{-1}$, is, however, about 25 s and is comparable with the evolution scale. The apparent downward propagation of coherent thermal structures is consistent with the stretching of symmetrical structures in the mean flow. After the evolution time t , an initially vertical structure would have an inclination of $\tan^{-1}[(t du/dz)^{-1}]$ or 53° and this is consistent with the tilt and direction of tilt of the contours in Fig. 2c. During the process of distortion of such a symmetrical structure by shear the x correlations are unchanged, but the z correlations are reduced by a factor $1/(1 + t du/dz)$, giving a ratio of scales, $1 + t du/dz$, of 1.76. The estimates of tilt and ratio scales are affected by the choice of $C = 0.6$, but show reasonable agreement with observations, indicating a shear-dominated structure at the 1-m scale.

The equation which governs the growth of small disturbances to a parallel shear flow in a stably stratified fluid is the Taylor-Goldstein equation². This has been solved numerically³ for given wave numbers, α , and Richardson number, J , using values of U , the horizontal current along the loch axis, and N , the Brunt-Väisälä frequency, obtained from 1-m vertical averages of the profiles (Fig. 3), with linear interpolation between the lowest measured values and the bottom of the loch. Figure 4 shows the conclusions. The largest growth rate is found at $J = 0.9$, $\alpha = 1.0$, corresponding to a wave of length 63 m moving with a speed which increases its amplitude exponentially in a time of 22.4 min. This wave requires a Richardson number only 10% less than that observed, that is an increased mean shear of only 5%. These waves would have their orientation across the wind and current direction (Fig. 5), and may account for the longer period fluctuations in temperature seen in Fig. 1. Inversions occurred most frequently in the regions of large rapid fluctuations (e.g. near 100, 500 and 900 s), and these appear to be the locations of the overturning billows. Similar waves have been observed using radar in the stable nocturnal atmospheric boundary layer over land^{4,5}, and may provide an important vertical diffusion process in stable boundary layer flows.

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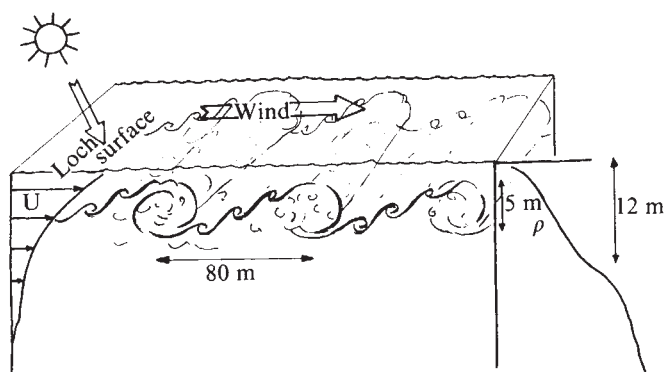
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Fig. 5 A schematic diagram of the billows. The vertical scale is inferred from the mean vertical profiles and the magnitude of the temperature fluctuations measured by the array.



The age and extent of the Red Sea oceanic crust

THE evolution of the Red Sea depression and the extent within it of oceanic crust have been widely discussed¹⁻³. It is generally accepted that the axial trough is floored by oceanic lithosphere formed at a constructive margin over the last 3-4 Myr by spreading at a rate of about 1.0 cm yr⁻¹ per flank^{2,6}. It is also clear from stratigraphic continuity and the trend of isofacial lines that the opening of the Red Sea started less than 45 Myr ago after a long period of tectonic and magmatic quiescence^{7,8}. Two areas of contention exist. First, how much of the Red Sea on either side of the axial trough is underlain by oceanic crust, and second, when was this oceanic crust formed?

The sedimentary record shows that before the Middle Oligocene (32 Myr) a shallow sea extended southwards from the main 'Tethyan Ocean' on to the then contiguous Nubian-Arabian plate^{8,9}. South of this sea was a low-lying flat or gently warped peneplaned land surface⁸. A drastic change occurred in the Red Sea at the beginning of the

Miocene (about 26 Myr). Evaporites began to be precipitated and these, by the end of the period (7 Myr), had attained thicknesses of 4–5 km. Also, coarse clastic sediments first appear as marginal intercalations within the evaporites^{10,11}. It is obvious that from that time (27 Myr) uplift occurred periodically along the sides of the Red Sea, which was attaining something near its present shape and extent. So, uplift and the formation of the Red Sea linear depression seem to have started about 26 Myr ago. At about 30 Myr ago the eruption of 50,000 km³ of trap basalts in Ethiopia and south-west Arabia started^{4,7} although localised, minor activity had occurred before that time¹². Both volcanism and uplift were active during the last 30 Myr although the main trap volcanism seems to have stopped by 24 Myr (ref. 13) before the major uplift took place⁴. Investigations in Afar^{14–18} have established the alternating nature of vertical movements and volcanism; when there was volcanism there was little or no uplift and vice versa. In the Afar the processes started about 25 Myr ago¹⁴ and have continued ever since with a cyclic periodicity of 2–4 Myr (ref. 16). Further investigation of the eastern Red Sea coast in southern Saudi Arabia⁴ convincingly demonstrate the presence of a 22-Myr old linear volcanic zone in which dyke injection is related to crustal thinning and warping and the development of the Red Sea depression in that area.

Are the geophysical data compatible with this primarily geological scenario? There is no geological objection to the conclusion, based on seismic refraction³ and magnetic anomaly^{6,19} studies, that the Miocene evaporites which flank the axial trough are underlain by oceanic crust, although it could be argued that the sub-evaporite P-wave velocities of 6.6 km s⁻¹ (ref. 3) are more characteristic of coarse-grained basic rocks of layer 3 than the finer-grained extrusives of layer 2. It is also geologically acceptable that the linear magnetic anomalies over the evaporites are oceanic strip anomalies whose amplitude has been reduced by the thick sedimentary overburden. Further, the near coast-to-coast fit of the Arabian plate to that of Nubia, based on plate tectonic theory, is in keeping with these data^{3,20}.

Girdler and Styles^{6,19} compared the anomaly profiles from an evaporite-covered area on the west side of the axial trough between 15° and 16°N with the magnetic anomaly reversal timescale. They conclude, on the basis of the best anomaly profile match, that the oceanic crust beneath these evaporites was formed either 41–34 Myr ago with a spreading rate of 1.3 cm yr⁻¹ per flank or between 29 and 24 Myr ago with a 2.3 cm yr⁻¹ spreading rate. They preferred the older period as the profile match was better and the spreading rate more plausible. It is geologically difficult to accept the older period as this requires that crustal down-warp, transcontinental rifting and the formation of over three-quarters of the Red Sea oceanic crust occurred at a time when there is no geological evidence of major uplift, magmatism and crustal deformation anywhere in the region. The younger period (29–24 Myr) would indeed coincide with an episode of intense magmatic and tectonic activity, but it still leaves 20 Myr (24–4) in which it is supposed that no spreading occurred under the Red Sea, whereas magmatic activity and large scale vertical and horizontal movements occurred around the periphery.

The model that best fits the geological data is a Red Sea linear depression that formed in the late Oligocene–early Miocene (25–26 Myr), with sea-floor spreading continuing intermittently ever since. I attempt here to determine whether the magnetic data can be fitted into this model. The problems arise from the evaporites. They are, on all accounts, of Miocene age and are primarily composed of halite with minor anhydrite, shale and marl horizons. Invoking the law of superposition, if the evaporites are

Miocene (26–27 Myr), then whatever is underneath must be pre-Miocene—older than 25 Myr. But must it? Evidence that this is not necessarily so comes from Girdler and Whitmarsh²¹ who describe DSDP sites 227 and 228 at the edge of the axial trough. Here, Miocene evaporites overlie oceanic crust confidently identified as aged less than 2.5 Myr on the correlation of the well-developed linear magnetic anomalies of the axial trough with the reversal time scale. They convincingly demonstrated that, as most of the evaporite is rock salt at temperatures above 100 °C at the base of the sequence, flow is not only possible but likely, and this is confirmed by petrographic studies (Shearman, personal communication). If Miocene evaporites have flowed over the recently formed axial trough oceanic crust, could not such flow have occurred throughout the development of the Red Sea? Recent volcanological²² and ocean-floor studies²³ indicate that sea-floor spreading can be intermittent on a relatively short time-scale. It would be consistent with the Afar evidence and the cyclic occurrence of coarse clastic sediments within the marginal evaporites, if spreading along the Red Sea constructive margin had taken place intermittently over the last 25–30 Myr. If the spreading phase continued long enough and was fast enough, the overlying evaporites would part and an axial trough form, only to be obliterated by salt flow when spreading stopped. Conversely, if the constructive margin processes were slow and short-lived, then oceanic lithosphere would be produced beneath an evaporite cover; a locale far more likely to form coarse-grained basic rocks with a characteristic 6.6 km s⁻¹ P-wave velocity³.

There are two possible objections to this model. First, of the 105 km of post-Cretaceous sinistral movement along the Jordan rift²⁴, only 40–45 km can be proven to be of post-Lower Miocene age. Yet Freund *et al.*²⁴ claim that this 40–45 km of latest movement probably took place in the last 12–7 Myr and, accepting an average movement rate of between 0.4 and 0.6 cm yr⁻¹ for the entire 105 km, they calculate that movement along the rift began 20–30 Myr ago (ref. 24, p. 124)—dates entirely in keeping with the proposals made here. Second, the ages of 36 and 30 Myr for a mildly metamorphosed argillite and basalt²⁵ at the bottom of two boreholes into the evaporites are quoted by Girdler and Styles^{6,19} in support of the 41–34 Myr spreading phase. But, as well as the dubious validity of stratigraphically uncontrolled whole rock K–Ar age determinations, Lowell and Genik identify a continental setting for both the argillite and the basalt (ref. 25, p. 249). It seems therefore that the proposal for a 41–34 Myr spreading phase must stand on magnetic anomaly matching alone. The veracity of this procedure for the Red Sea must be queried, for it produces results that conflict with the majority of other evidence.

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Andesitic pyroclastic flows from Colima Volcano

COLIMA (19°31'N, 103°37'W) is important as one of the most continuously active continental andesite volcanoes in North America. Various types of pyroclastic and lava eruptions have been reported over the last 400 yr (ref. 1). Here we describe the eruption of andesitic lava and associated pyroclastic flows during December 1975–April 1976. On the basis of our visits between January and June, 1976, we comment on the relationship of this to earlier eruptions of Colima.

Colima is a large composite volcano, now slightly over 4,000 m high, situated within a caldera (5 km diameter) on the southern flank of the extinct Pleistocene volcano of Nevada de Colima (4,320 m, rising from a base level of 1,500 m). Colima forms part of the Mexican Volcanic Belt, which is associated with subduction of the oceanic Cocos plate below continental Mexico². Eruptions of Colima have been noted since 1576 and to view the 1975–76 eruption in perspective we summarise the history during the present century.

In 1906, the volcano had a conical form, with truncated summit and a small crater. A large explosive eruption in 1913 produced an extensive airfall deposit and 'nuée ardentes'^{3–4}, and left a much larger crater with a rim height of 3,960 m (ref. 4). Growth of a lava dome within the crater during 1913–35 was accompanied by mild explosive activity, and by 1935 the dome was 60 m below the crater rim. The volcano was quiescent until 1958, when the dome began to rise again so that its highest parts reached the level of the crater rim. This led to extrusion of a blocky andesite flow from the side of the dome down a prominent gully on the northern flank of the cone during 1961–62 (ref. 5; Fig. 1). Since 1962 the dome has grown slowly so that the volcano's height increased to an estimated 4,100 m by 1975, before the start of the latest eruption.

The eruption started on December 11–12 with mildly explosive activity, the extrusion of andesite lava in three small lobes to the north and two flows down the south-east flank of the cone (Fig. 1). Extrusion of the lobes was completed rapidly, probably within a few days, and they were static on January 3. On that date the east-south-east flow was moving very slowly but the most south-easterly flow was very active and continued to flow until studied in detail on January 14–15. In contrast with the lobes to the north of the cone, and the older 1961–62 flow, both south-easterly flows have prominent levées.

A variety of pyroclastic and rock deposits, below the north lobes and on the western side of the volcano below the summit dome, are associated with the early phase of the eruption. These include 'hot avalanche' deposits⁶ erupted during lava or dome growth on a steep slope, colder avalanche deposits formed during cooling and disintegration of lava, and more slowly accreted rock-fall debris. During part of the early phase, juvenile andesite blocks up to 0.5 m³ were ejected explosively, falling up to 2 km from the cone.

Between January 3 and 15 there was strong fumarole activity from depressions in the summit dome (Fig. 1), occasional colder rock-falls, and active lava flow movement only on the most south-easterly flow. The lava flowed down the Barranca de la Arena (Fig. 1) and reached 2 km from the cone by January 14–15, when it was examined in detail at the point indicated on Fig. 1. At this time andesite lava was flowing down the barranca over a break of slope 50 m high. Flow of lava down the valley was accompanied by pyroclastic flows ranging from hot avalanches to a nuée ardente type of flow. The hot avalanches comprised large falls of hot (often flowing) rocks with total volumes up to 10 m³. The falls were from the steep, 40-m front face of the lava flow. The slope below this face was about 40°, this slope representing a steep section of the barranca, smeared with lava debris. The avalanches generally travelled up to 100 m down the valley and recurred every 4–5 min.

All gradations occurred between the hot avalanches and a pyroclastic flow resembling a small nuée ardente. The latter flow was initiated by the collapse of a substantial mass of hot (often glowing) blocks from the lava flow front. These were immediately engulfed in an expanding dusty cloud. After formation, the flow moved away from the lava front quietly (in great contrast to hot avalanches). The cloud expanded simultaneously in all directions away from the flow, eventually engulfing a considerable area (hundreds of m²) of the surrounding countryside and leaving a deposit of very fine brown dust. The cloud had cooled

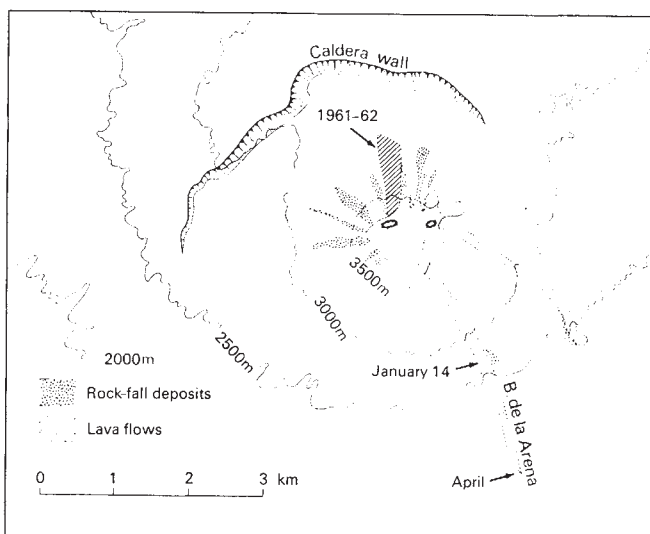


Fig. 1 Map of Colima volcano, showing lava and rock fall (including pyroclastic deposits) formed during the 1975–76 eruption. The outlined area labelled, 1961–62, extending to the north of the summit, is the lava flow erupted during these years. Solid lines on the summit enclose depressions in the lava dome which have abundant fumaroles.

before engulfing the observation point, 200 m from the flow front.

We believe that the quietness of the pyroclastic flows is significant since it must indicate movement of blocks as a suspension within the flow. Arrest of the flow was accompanied by resumption of considerable noise, and blocks were deposited from the flow. The pyroclastic flows were also characterised by a narrow channel, a few m wide, in the underlying rock deposits. This was partly erosional in character but also reflected alignment of the deposited material. Such pyroclastic flows were formed every 7–8 min during the period, the earlier passage of flows down the same valley being indicated by destruction of trees at the top of the valley walls—the trees having been aligned down-valley. Between January 15 and April the lava flowed a further 1–2 km (Fig. 1) and movement ceased on June 20.

This type of pyroclastic flow has been reported from several volcanic areas, including Indonesia and central America (for example, Santiaguito, Guatemala¹⁰), and is similar to flows erupted at Merapi (Java) as a result of collapse of the side of a growing lava dome^{7,8}. They have been termed 'Merapi-type' pyroclastic flows⁹. The pyroclastic flows were a common and characteristic part of the 1975–76 eruption of Colima, and we infer that they grade upwards in scale to larger nuée ardentes described from earlier eruptions of Colima and possibly formed at an earlier stage of the 1975–76 eruption. It seems likely that deposits from these eruptions would be difficult to identify with confidence in an older mixed succession of lavas and pyroclastics. We therefore suggest that such pyroclastic flows are more important than commonly supposed in the growth of composite andesite volcanoes. Although it is difficult to estimate, our study of Colima suggests that as much as a third of the erupted products of this volcano might be deposits from pyroclastic flows of the types described here.

Our studies of Colima were made during a visit sponsored by Earthwatch (Educational Expeditions International) and the Centre for Field Research (Belmont, Mass.). We thank the expedition participants for help in making the observations, E. Areola and I. Flores for assistance in Ciudad Guzman and on Colima, and many friends in Mexico for help and encouragement.

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Detection of atmospheric infrasound by homing pigeons

ACOUSTIC waves of frequencies below 10 Hz are common in the atmosphere. They are generated by various sources, including wind, thunderstorms, weather fronts, magnetic storms, aurorae, ocean waves, earthquakes, and many of man's mechanical devices^{1–3}. Many of these atmospheric oscillations are of high amplitudes (frequently well above 100 dB SPL at frequencies below 5 Hz, and above 120 dB below 1 Hz) but because they are outside the normal limits of human hearing they usually go unnoticed (although humans can detect extremely loud artificial infrasounds generated in test chambers, these sounds are louder than most natural infrasounds and verge on the threshold for pain)^{4,5}. It has been suggested, however, that migrating and

homing birds might make use of the cues provided by such infrasounds⁶. During our ongoing study of the sensory basis of avian orientation, we investigated this possibility. We report here that homing pigeons (*Columba livia*) are sensitive to infrasounds at reasonable amplitudes.

Our testing procedure, using a conditioned cardiac response (increased rate of beating), was very similar to one used to test for sensitivity to barometric pressure changes⁷. The pigeon was fitted with two ECG surface electrodes and two shock electrodes (implanted under the skin of the breast), restrained by a leather harness, and placed in a sound-insulated conditioning chamber 30×44.5×30 cm high (the chamber was described more fully in the barometric pressure study⁷). Fresh air for respiration was supplied at 5 l min⁻¹ through a system of pressure and vacuum pumps; however, this input was cut off and the chamber completely sealed for short periods while tests at very low frequencies were run (this reduced the background noise in the chamber by about 20 dB).

Two methods of producing acoustic pressure oscillations were used. In some of the early tests, we used a motor-driven piston of approximately 1 cm diameter, with an adjustable stroke to produce changes in amplitude. This apparatus was modified from a Phipps and Bird small animal respiration pump, model 70-878-01. The pressure output was a sine wave and the frequency was varied by changing the motor speed. The piston was always stopped at the mid-point in the cycle, insuring that the next stimulus would start at zero amplitude. In later experiments, the pressure variations were produced by a loudspeaker driver (Atlas sound PD-60-T) connected to the chamber with tubing so as to act on a closed volume of air. In such a closed system, the loudspeaker driver was not limited to the normal free-air frequency response, but instead could produce low-frequency pressure changes in the chamber well below 0.1 Hz. The loudspeaker driver was powered by a Hewlett-Packard sine wave generator, model 200 C. With both methods of producing pressure changes, the sounds were piped through approximately 2 m of plastic tubing 1 cm in diameter and passed through a mechanical lowpass filter before entering the chamber. The filter served to reduce high-frequency sounds in the audible portion of the spectrum. The lowpass filter was constructed of a cylinder 5 cm in diameter and 15 cm long, coaxial with the 1 cm tubing and packed with glass wool. The frequency characteristics of the filter will be described in detail elsewhere. The rise and fall times of the stimulus envelope were controlled to virtually eliminate high-frequency transients.

Stimulus presentations were at random intervals. Before each presentation, the heart rate of the pigeon was monitored for 5 s; this was followed by a 10 s stimulus, which was in turn followed immediately by a shock (1 mA for 350 ms from a 60 Hz a.c. constant-current source). After a few trials, a conditioned response developed, and the heart rate increased each time the bird detected the stimulus, before the shock. For a positive response to be counted, the heart rate during the 10 s stimulus period must have exceeded by at least 12 beats per min the highest rate recorded during the preceding 5-s baseline period. Typical baseline rates were approximately 140 beats per min, and accelerations in response to stimuli ranged from 30 to 60 beats per min. Instantaneous heart rate was monitored with a Grass Instrument 7P4 tachograph, with a linear voltage output proportional to heart rate, measured on a beat-to-beat basis. A permanent record of the heart-beat rate was made on a Sanborn 350 chart-recorder.

Our experiments demonstrate that homing pigeons can detect sound energy below 10 Hz at amplitudes well within the range of naturally occurring sounds at these frequencies. Indeed, they can detect frequencies below 1 Hz. At 113.8 dB r.m.s. SPL (1 mm H₂O), most birds responded to stimuli of 1.67 Hz and 0.8 Hz at a much higher rate than background

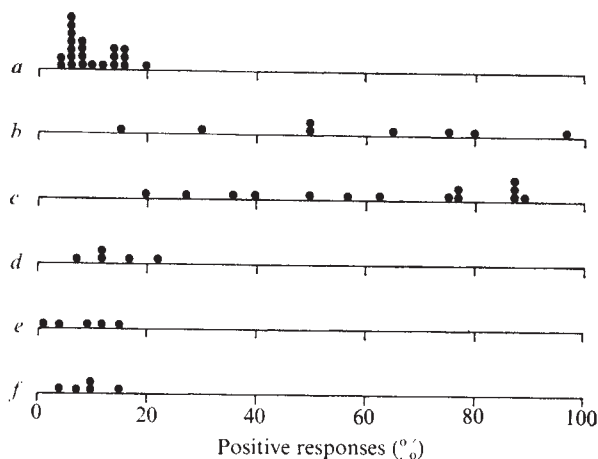


Fig. 1 Percentage of positive responses of individual homing pigeons to infrasound stimuli (40 presentations each). *a*, Chance 'positive' response levels of 22 birds when no stimulus was provided; this is the background level against which responses in other test situations should be evaluated. *b*, Responses of eight pigeons to sounds of 1.67 Hz. *c*, Responses of 14 pigeons to sounds of 0.8 Hz. *d*, Responses of five pigeons to sounds of 1.67 Hz when the tubing between the sound generator and the test chamber was closed by pinch clamps. *e*, Responses to sounds of 1.67 Hz by five pigeons whose cochleas and lagenas had been removed. *f*, Responses to sounds of 1.67 Hz by five pigeons whose columellas had been bisected. All sound amplitudes were 113.8 dB rms SPL.

(compare Fig. 1*b* and *c* with 1*a*). The response could be eliminated if two pinch clamps were placed on the tubing between the sound generator and the test chamber (Fig. 1*d*).

From preliminary experiments, we estimate the threshold (50% response) to be approximately 80 dB at 1.5 Hz, 60 dB at 5 Hz, and 50 dB at 10 Hz. These compare with published values for human thresholds of 132 dB at 1.5 Hz, 115 dB at 5 Hz and 104 dB at 10 Hz (ref. 5). The ambient noise levels inside the insulated chamber were 70 dB at 1.5 Hz (band width (bw) 10% of centre frequency), 50 dB at 5 Hz (bw 1%) and 47 dB at 10 Hz (bw 1%); inasmuch as the 50% threshold values were only slightly above these noise levels, it seems possible that the pigeons would be even more sensitive to signals of these frequencies if tested in a quieter chamber. (A more detailed study of threshold response values, down to 0.1 Hz, is in progress.)

To determine the location of the receptors responsible for infrasound detection, we tested five pigeons from which the cochleas (and lagenas) had been surgically removed, and also five pigeons with intact cochleas but with both columellas bisected so that there was no direct conduction of sound between the tympanum and the oval window. Figures 1*e* and *f* show that neither of these groups of birds respond to an infrasound stimulus of 1.67 Hz at 113.8 dB; the response levels for both groups were indistinguishable from those of normal birds in the absence of a stimulus. These results suggest that the receptors for infrasound are in the inner ear, but it is not clear which organ is responsible because removal of the cochleas and lagenas unavoidably damages the remaining portions of the labyrinth, one part of which has recently been shown to function (at least in frogs) as an auditory receptor⁴.

Unlike sounds easily audible to humans, atmospheric infrasounds can travel huge distances—often thousands of miles—without much attenuation (which is proportional to the square of the frequency). Hence such sounds could potentially provide birds with much useful information. For example, the ability of birds to sense approaching weather fronts might be aided by detection of infrasounds funnelled to earth by those fronts or by detection of the infrasound components of thunder associated with storms that are still hundreds of miles away. Localisation of several remote infrasound sources could serve as a distant landmark system for triangulation of the home site and of

the location of a release site. Properly utilised, infrasound information could thus assist in almost every aspect of avian navigation, in both homing and migration.

Whether one or more of these potential uses is actually realised is not known. There are several reasons why birds might have difficulty extracting meaningful information from infrasounds. For example, they would need to distinguish between spatially coherent infrasounds and so-called pseudosounds, which are very local pressure oscillations generated by winds and atmospheric turbulence. Further, the usual binaural comparison methods of localising sounds would be ineffective where the wavelengths are so very long (approximately 300 m for a sound of 1 Hz). Neither of these problems should be insurmountable for a bird in flight. Thus a flying bird might be able to reconstruct the spatial patterns of atmospheric infrasounds and thereby separate them from local pseudosounds. Quine and Konishi⁹ have shown that, in the usual audible range, owls can distinguish frequencies that differ by as little as 0.5–0.7%; if such discrimination holds for pigeons in the infrasound range, then doppler shifts in frequency, which for a pigeon flying at 20 m s⁻¹ toward or away from the sound source would be as high as 14%, could provide ample information for localisation. Only further research can reveal whether or not this is mere speculation.

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Genetic influences and infantile autism

IN his original description of infantile autism, Kanner suggested an "inborn defect", because symptoms were often present from early infancy. Despite the rarity of a family history of autism and lack of a known increase in parental consanguinity, there are two reasons for suspecting hereditary influences: the 2% rate of autism in siblings is 50 times that of the general population¹, and a family history of speech delay is found in about a quarter of families². Reports of single pairs of twins with autism have not added much to our knowledge of genetic effects because of a bias toward reporting monozygotic (MZ) concordant pairs and because few reports contain both adequate clinical descriptions and evidence of zygosity¹. We therefore undertook a study of a systematically collected sample of 21 pairs of same-sexed twins, one or both of whom had autism as diagnosed by the criteria of Kanner³ and Rutter⁴. The

results reported here indicate the importance of hereditary influences in the aetiology of autism.

The names of twins were collected from consultants, schools and units for autistic children, the National Society for Autistic Children and two hospital twin registers. Apart from two pairs in which dermatoglyphics were used, zygosity was determined by blood grouping in all cases not markedly different in physical appearance. Case notes of the 33 pairs thus obtained were reviewed and eight pairs were rejected on diagnostic grounds. The remaining 25 pairs and their families were extensively interviewed, tested and examined at home or in hospital. Obstetric records and case notes were also studied. On the basis of all information, a further four pairs were excluded as not meeting diagnostic criteria.

The adequacy of our sampling is shown by the MZ:DZ ratio (11:10) which was about that expected for same-sexed twin pairs surviving the first year (6:7); and by the number of autistic twin pairs found. In the English twin population of school age who would meet our diagnostic criteria, between 19 and 27 autistic children would be expected and 20 were found. Our sampling of children over school age was less complete, but not likely to be biased as to zygosity or concordance since most cases came from twin registers.

The diagnoses were made from case summaries, numbered randomly to prevent sorting by pair, and from which name, exact age, and zygosity had been deleted.

At least one member of 21 same-sexed pairs of twins met the diagnostic criteria for autism. In all, the 11 MZ and 10 DZ pairs gave rise to 25 autistic children. Apart from being twins, these were similar to other reported series of autistic children with respect to sex ratio (3.4:1), social class (57% from I and II), and IQ (about 1/2 severely retarded but 1/4 with normal non-verbal IQ). Affective disorder was described in four families, but schizophrenia did not occur. One sibling suffered from autism (a rate of 2.8%). Delayed speech was reported in three families (Table 1).

Four of the 11 MZ pairs but none of the 10 DZ pairs were concordant for autism ($P=0.055$). All concordant pairs were male. Because autism is a rare condition (about 4 per 10,000 children), the MZ concordance rate is equivalent to a very high correlation in liability (over 0.9) using the multifactorial model^{5,6}.

If, using this model, it is hypothesised that autism reflects some form of continuously distributed, abnormal characteristic, the question arises whether the non-autistic co-twins showed any other abnormality. We found that very few did in the DZ pairs but in most MZ pairs the co-twins showed

Table 1 Pair-wise concordance by zygosity

	MZ pairs (<i>n</i> = 11)	DZ pairs (<i>n</i> = 10)	MZ-DZ difference (exact test)
Concordance for autism	36%	0%	$P = 0.055$
Concordance for cognitive disorder (including autism)	82%	10%	$P = 0.0015$

some form of cognitive disorder (see Tables 1 and 2).

Thus, in addition to the autistic twins, 6 non-autistic co-twins (5 MZ and 1 DZ) showed a cognitive abnormality as defined by the criteria of no phrase speech until 3 yr (three cases), IQ 70 or below (two cases), severely abnormal articulation after 5 yr (two cases), or scholastic difficulties requiring special schooling (two cases). Four non-autistic twins showed only one of the above features and none were severely handicapped. All the autistic twins met at least two of the criteria. Thus, 9 (82%) of the 11 MZ pairs were concordant for cognitive disorder/autism compared with only 1 (10%) of the DZ pairs ($P=0.0015$). The findings

Table 2 Summary of findings for MZ pairs

	First-born twin	Second-born twin
1	Autism + severe MR	Autism + severe MR
2	Autism + mild MR	Autism
3	Autism + severe MR	Autism + severe MR
4	Autism	Autism + mild MR
5	Mild MR	Autism + severe MR
6	Autism	Educational difficulties (ESN school)
7	Severe articulation defect	Autism
8	Autism + severe MR	Language disorder
9	Mild MR	Autism + severe MR
10	Autism + mild MR	Normal
11	Normal	Autism

MR, Mental retardation.

ESN, Educationally subnormal.

strengthen the suggestion of genetic determination and indicate that what is inherited is a form of cognitive abnormality which includes but is not restricted to autism.

Twins have a raised rate of perinatal difficulties leading to brain injury and it was necessary to check that the concordance was not an artefact of these. We found that it was not. In none of the four pairs concordant for autism did both twins have a history of brain injury. Furthermore, no twin with cognitive disorder in the absence of autism showed evidence of perinatal brain injury (Table 3).

Brain injury was, however, relevant to discordance

Table 3 Summary of biological hazards in discordant pairs

Hazard	Autistic twin	MZ pairs	Non-autistic twin
Definite	Multiple congenital anomalies		—
	Neonatal convulsions		—
Possible	Severe febrile illness		—
	Pathologically narrow cord		—
None	—		—
	—		—
	—		—
		DZ pairs	
Definite	Apnoea		—
	Delay second birth		—
	Delay second birth		—
	Delay second birth		—
Possible	Severe haemolytic disease + apnoea		Delay second birth
or			
Difference in severity	Severe haemolytic disease + apnoea		Mild haemolytic disease
	Birth weight 1 and $\frac{1}{4}$ pounds lower		—
	Birth weight 1 and $\frac{1}{4}$ pounds lower		—
None	—		—
	—		—

regarding autism. We identified five features known to be associated with brain injury: kernicterus, perinatal apnoea of more than 6 min, neonatal convulsions, multiple congenital anomalies, and a second birth delayed by at least 30 min. In 6 of the 17 pairs discordant for autism, the autistic child but not the non-autistic co-twin had experienced one or more of these biological hazards in the perinatal period.

A wider definition of biological hazard was then applied to the 11 cases not differentiated on the stricter criteria. Using the wider definition (lower birth weight by 1 pound or more, pathological narrow umbilical cord, a more severe kernicterus with apnoea and a severe febrile illness—possibly encephalitis—just before onset), a further six discordant pairs were differentiated. Thus, in 12 of 17 pairs discordant for autism, the autistic member probably or possibly suffered a brain injury, whereas in none of the discordant pairs did this occur only in the non-autistic member.

To summarise the results: the markedly higher rate of concordance in MZ, compared with DZ, pairs for both autism and cognitive abnormalities indicates the importance of genetic factors in the aetiology of autism. Indeed, the concordance rates in conjunction with the very low prevalence of autism in the general population point to a strong genetic determination. Nevertheless, the finding of an association between biological hazards in the perinatal period and autism also demonstrates the important role of environmental influences for the causation of autism (but not cognitive disorder).

In some cases, genetic factors seem to be both necessary and sufficient causes. Among the eight autistic children in the four concordant MZ pairs, biological hazards (even using the broader criteria) occurred in only one child. He was more severely affected than his twin. On the other hand, brain injury alone seems to be a sufficient cause in some cases. In one of the two completely discordant MZ pairs, the autistic twin had experienced neonatal convulsions, whereas the non-injured co-twin was normal in every way. The importance of brain damage is also indicated by the finding in other studies of autism in children with congenital rubella or infantile spasms.

Finally, some cases seem to result from the combination of an inherited cognitive defect plus brain damage. In three of the five MZ pairs discordant for autism but concordant for cognitive deficit, the autistic child had experienced a biological hazard whereas the cognitively impaired non-autistic co-twin had not.

Determining the mode of inheritance is difficult since autistic children rarely reproduce. It would be helpful to know what happens to the offspring of the non-autistic twins with cognitive impairment, but no such information is available.

In summary, we conclude that this systematic study of 21 same-sexed twin pairs in which at least one twin showed the syndrome of infantile autism, indicates the importance of a genetic factor in the aetiology of autism. It also indicates the importance of brain injuries, especially during the perinatal period, which may operate either by themselves or in combination with a genetic predisposition involving language. Both the mode of inheritance and exactly what is inherited remain uncertain.

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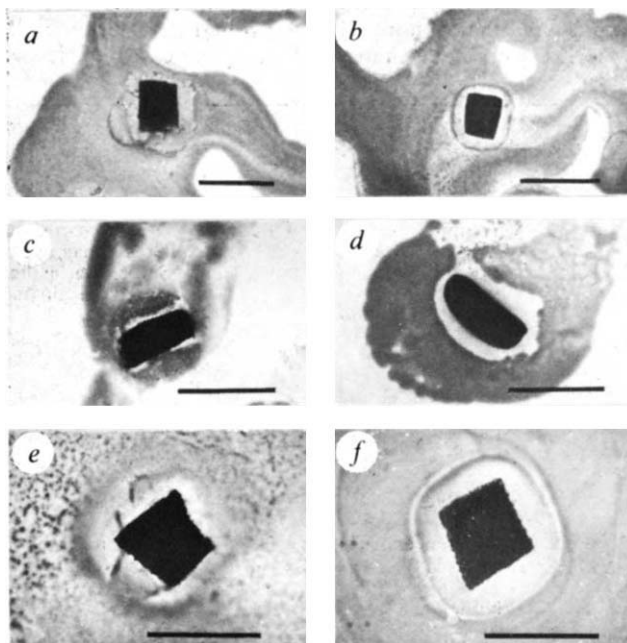
Anti-fouling role of antibiotics produced by marine algae and bryozoans

A GRADIENT of antibacterial activity along fronds of the large brown seaweed *Laminaria*¹ runs opposite to gradients of density of bryozoans and spirorbid tube worms settled on the fronds^{2,3}. Bryozoan colonies attached to a *Laminaria* frond grow towards the younger region⁴, where antibiotic production is lowest. It has been suggested that tannins produced by branch tips of *Sargassum* have an anti-fouling role⁵, but we have seen no other clear evidence of such an effect, although many marine plants and animals remain remarkably free from encrustation by other organisms⁶. We have now found further evidence in several examples of differential settlement by spirorbid worms on algae and by a bryozoan on another species of bryozoan.

We had noticed that holdfast branches of *Laminaria digitata* (Huds.) Lamour on Pembrokeshire and Plymouth shores bear populations of *Spirorbis inornatus* L'Hardy and Quévieux, always attached to the sheltered inner sides. The same spirorbid settles abundantly on *Himanthalia elongata* (L.) S. F. Gray, but only on the undersides of the button-like expansions which protect the attachment points of the alga. In laboratory experiments, which followed the usual procedures⁷ and will be reported in detail elsewhere, we found that settling *Spirorbis* larvae invariably select those parts of the two algae, irrespective of the contours of the surfaces and of their orientation to light and gravity. We tested these two sites for differential production of antibiotics.

Disks 5 mm in diameter cut from the surfaces were tested by standard procedures⁸ against *Staphylococcus aureus*, strain NC1B8244. This grew readily or formed indistinct clouded zones around surfaces normally shaded and colonised abundantly by epibionts (Fig. 1a and c), but left

Fig. 1 Growth (a, c and e) and inhibition (b, d and f) of *Staphylococcus aureus* round pieces cut from inner surface of *Laminaria* holdfast branch (a); outer surface of latter (b); lower surface of *Himanthalia* button (c); upper surface of latter (d); growing edge of *Fustra foliacea* colony (e); old part latter (f). The scale lines represent 5 mm.



clearer and wider zones of inhibition around those normally exposed to light and unfavourable for the settlement of *Spirorbis* larvae (Fig. 1b and d).

We similarly tested the frondose bryozoan *Flustra foliacea* (L.), which is readily colonised by the bryozoan *Scrupocellaria reptans* (L.), with the initial settlement of larvae concentrated around the distal growing edge of the colony^{4,9}. We found that region to be virtually devoid of antibacterial activity, whereas clear zones of inhibition usually appeared round pieces from older parts of the fronds (Fig. 1, compare e and f). The differences between pairs in each of the three experiments illustrated in Fig. 1 were all highly significant (Table 1).

Table 1 Mean widths of inhibition zones around 5-mm disks

Species	Region	No. of replicates	Mean width (mm) $\pm$ s.e.	P
<i>Laminaria</i>	Holdfast inner side	16	0.81 ± 0.13	< 0.01
<i>Laminaria</i>	Holdfast outer side	16	1.59 ± 0.15	
<i>Himanthalia</i>	Button lower side	24	0.28 ± 0.09	< 0.01
<i>Himanthalia</i>	Button upper side	24	1.00 ± 0.10	
<i>Flustra</i>	Growing edge	24	0.35 ± 0.11	< 0.01
<i>Flustra</i>	Old part of frond	24	1.35 ± 0.35	

Pieces cut from older parts of *Flustra* fronds, kept in a stoppered tube, emitted for several days a characteristic smell of lemons, whereas similar pieces from growing edges had a different smell and soon decomposed. This deserves further study, considering the insect-repellent reputation of citronellal, the apparent distaste for citronella grass shown by grazing buffalo¹⁰ and the antiseptic properties of some monoterpene derivatives. The suggestion that tannins are the active agents in algae is supported by the finding that polyphenol production in *Laminaria* holdfasts seems to be increased at higher illuminations¹¹.

The settlement behaviour and growth orientation noted would confer advantages on the epibionts, ensuring a longer lifespan on *Laminaria* fronds, less competition for space on newly formed surfaces and greater protection from sand inside holdfasts or under *Himanthalia* buttons. Populations of *Spirorbis inornatus* are to some extent adapted to secure such advantages, for most larvae from parents on *Laminaria* settle on *Laminaria* rather than on *Himanthalia* (our unpublished results) whereas most from *Himanthalia* parents show the reverse preferences. But larval settlement on the insides rather than the outsides of *Laminaria* holdfasts, and on the lower rather than the upper sides of *Himanthalia* buttons, is not invariably the result of natural selection for avoidance of abrasion by sand, for similar behaviour is shown by larvae derived from a population of *Spirorbis spirorbis* L. at Swansea, which is confined to *Fucus* in pools 30 m from the nearest *Laminaria* (which is never colonised by *Spirorbis* in that locality) and 30 miles from the nearest *Himanthalia*. There could have been no recent evolutionary adaptation of that population to *Laminaria* or *Himanthalia*. The algae, on the other hand, presumably benefit from keeping their exposed surfaces clear for photosynthesis and there seems to be little need to discourage settlement on the sheltered surfaces inside holdfasts and under *Himanthalia* buttons. The antibiotics of *Flustra* and *Laminaria* seem to discourage only larvae, and are not obviously deleterious to older epibionts, which grow readily, no doubt protected by their greater size. The settlement on areas of new growth is not due to inability of growing regions to produce antibiotics, for several species of algae show maximum antimicrobial activity in regions which grow fastest^{1,5}. In those species the growth regions are distal, suggesting that the antibiotics are involved in protection against epiphytes and macrograzers, as well as against the pathogenic invasions which would tend to follow damage by abrasion.

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Cell lineage analysis of germ cells of *Drosophila melanogaster*

THE initial events of differentiation of genetically identical nuclei within the eggs of higher organisms are generally believed to be triggered by a heterogeneous distribution of substances within the egg^{1,2}. One of the clearest examples of such differential distribution of determinative substances concerns the posterior polar plasm of some insects. Morphological observations suggest that all cleavage nuclei in such insects have identical developmental potential, and that only the few nuclei that chance to migrate to the posterior polar region and thus to include a sufficient quantity of the morphologically distinguishable polar plasm, acquire the capacity to give rise to the germ line³. Various experimental approaches⁴⁻⁸ have provided very strong, although not compelling⁹ evidence for such a causal relationship between the polar plasm and germ line differentiation. Genetic fate mapping experiments described here strongly suggest that the germ cells of *Drosophila melanogaster* originate from the posterior-most region of the blastoderm, and they unequivocally rule out the possibility that the germ cells originate from a mid-dorsal position¹⁰. The posterior-most location of the germ cells supports, or at least is consistent with, the notion that the polar plasm determines whether or not a given cleavage nucleus will acquire the capacity to differentiate into a germ cell.

A priori, two possibilities can be distinguished. The first, as outlined above, states that "any nucleus may become a polar nucleus if it happens to move into the region of the posterior polar plasm, regardless of cell lineage" (ref. 3). The second assumes that the prospective germ cells are set aside during early cleavage before their interaction with the posterior polar plasm or with any other cytoplasmic region. One critical test between these two alternatives involves genetic determination of cell lineage relationships of the primordial germinal nuclei: only the first requires that they are mapped to the area of the blastoderm that contains the posterior polar plasm. The reason for this prediction is apparent, for example, from the observation that one ancestral nucleus in the posterior pole region can give rise to one daughter cell which contains the visible polar granules and one which does not³, and hence that presumably only the former is likely to contribute to the germ line.

The technique of genetic fate mapping^{11,12} provides one approach by which cell lineage relationships of various anatomical regions can be determined⁸. Using this technique, Falk *et al.*¹⁰ located the gonads on the genetic fate map of *D. melanogaster* "about half way along the blastoderm near the mid-ventral line". They suggested therefore that "the assumption that the site at which a nucleus lands in the periplasm decides its fate has to be modified, at least for nuclei destined to give the gonads" (ref. 10).

Both the observations that some pole cells in *D. melanogaster* give rise to somatic cells rather than primordial germ cells¹³, and the observation that in the wasp *Pimpla turionellae*, although characteristic pole cell formation and migration occur, germ cells are found in the absence of pole cells^{14,15}, seem to be compatible with this suggestion. But two other attempts^{16,17} have been made to determine the developmental origin of the gonads by means of genetic fate mapping, and these gave a posterior position compatible with the hypothesis that the site at which a nucleus lands in the cytoplasm decides its fate. These inconsistent results are surprising in view of the reproducible interrelationships between cuticular landmarks that are reflected by many independently derived maps^{11,12,16-23} and in view of the strong evidence for the validity of the fate mapping procedure^{12,21,24}.

All three investigations mapped whole gonads either at the larval stage^{10,17} or at the adult stage¹⁶. It is well established that the gonads of *Drosophila* derive from two developmentally unrelated components: the germ cells which give rise to the germ line and mesodermal cells which give rise to the gonadal sheath and interstitial cells^{8,25}. The nature of the interaction between these two components is unclear^{13,25}. In addition, the attachment of testes in adult gynandromorphs to the developmentally distinct female oviducts causes degeneration of the testes²⁶. These theoretical considerations, coupled with the technical difficulties encountered in fate mapping of internal organs and the need to use a functional, rather than morphological criterion for the germ nuclei, make it very difficult to resolve these contradictory results and their bearing on the hypothesis that the posterior polar plasm contains germ cell determinants. A clearcut resolution of this problem is therefore likely to emerge only from functional mapping of the germinal nuclei themselves, irrespective of other elements of the reproductive system⁸. The experiments described here constitute the first clearcut genetic evidence for the developmental origin of the germ cells, and they show that the germ cells of *Drosophila* do indeed originate from the general area that has been traditionally assigned to them by cytologists—the posterior tip of the egg.

Males of the constitution $y-y^+ Y; pal-pal$ were crossed to $XY, y B$ females^{20,27}. The sons from this cross were screened for *pal*-induced somatic mosaicism for the extra Y chromosome; this can be detected by the appearance of yellow and non-yellow cuticular patches in the same male. The frequency of mosaicism among the F_1 males has not been calculated directly, but ranged approximately from 0.001 to 0.002. On average, each cuticular region was yellow in 40% of the mosaic males, non-yellow in 55% and mixed in the remaining 5%. The mosaic distribution of the entire cuticle of each rare mosaic male was recorded. Subsequently, each mosaic male was crossed to five to nine yellow females. These yellow females were kept in a stock in which all males carried the B^Y chromosome and thus any non-virgin yellow female could be detected from a phenotypic examination of her sons. The constitution of the germ cells of each 0–14-d old mosaic male was inferred from the phenotype of his sons: the germ cells of a male that only gave rise to non-yellow and fertile sons must have contained the extra marked Y chromosome; yellow and sterile sons indicate that they did not contain this extra Y chromosome, and when both phenotypes were found among the male progeny of a single male it could be assumed that such a phenotypically mosaic male was also germinally mosaic.

Out of a total of 260 phenotypically mosaic males obtained, six died shortly after their isolation and gave no offspring, 17 were sterile, six gave rise to less than 62 sons and 16 gave rise to some haplo-4 progeny. Of the remaining 215 mosaic males, 85 gave rise to y sons, 94 gave rise to y^+ sons and 36 to both y and y^+ sons. An average of 312 sons was observed per mosaic male and the smallest number of sons scored per male was 62. The data obtained from these 215 males made it possible to correlate the constitution of the germ cells to various cuticular landmarks and thus to determine their position on the genetic fate map of *D. melanogaster* (Fig. 1).

To rule out any sex-specific differences in the developmental origin of the germ cells and to obtain an independent corroboration of their posterior-most position (Fig. 1), an analogous

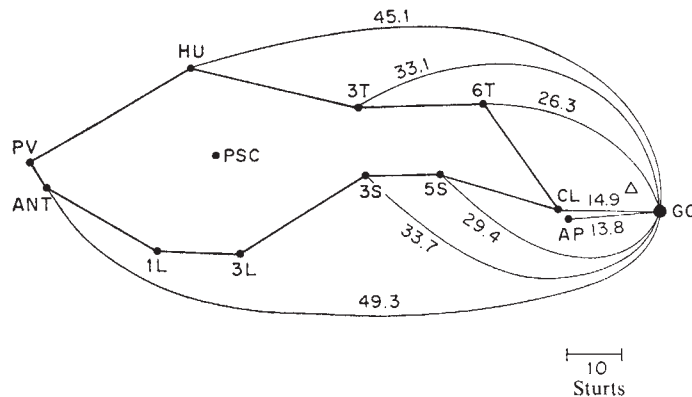


Fig. 1 Genetic fate map of the right half of *D. melanogaster* blastoderm, with special reference to the position of the germ cells. Because it was not possible to rule out *a priori* a bilateral symmetrical origin of the germ cells, data from germinally mosaic males were not included in the calculations of distances between the germ cells and various cuticular landmarks. But the general outline of the map, and, in particular, the posterior position of the germ cells, is preserved when the data from germinally mosaic males are also included or when the map distances are computed according to the suggested algebraic correction^{19,22} for systems in which the mean proportion of mutant tissue is significantly different from 0.5. The open triangle represents the position of the germ cells triangulated on a map derived from 105 mosaic females and then superimposed on the map of 215 phenotypically mosaic males shown here. ANT, antenna; AP, anal plate; CL, clasper; GC, germ cells; HU, humeral bristles; PSC, posterior scutellar bristle; PV, post-vertical bristle; 1L, first leg; 3S, third hemisternite; 3T, third hemitergite.

investigation has been carried out with mosaic females. Males of the constitution $y/y^+ Y; pal/pal$ were crossed to attached $X C(1)RM, y pn v/0$ females. The automatic virgin daughters obtained from this cross were screened for somatic mosaicism and their pattern of mosaicism was recorded. Subsequently, these mosaic females were crossed to yellow males. A total of 116 Y-chromosome mosaic females was obtained. The analysis, however, was based only on 105 mosaic females that gave rise to a minimum of 22 sons. The mean number of sons per female in this group was 98. Again, a fate map of cuticular landmarks was constructed and the location of the germ nuclei on this map was determined. Although there were some differences between this map and the map derived from mosaic males (Fig. 1), they were very similar in general outline and hence the map obtained from these 105 mosaic females is not reproduced here. Instead, the position of the germ cells in this map is superimposed on the map shown in Fig. 1.

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Cell lineage in retinal development of mice studied in experimental chimaeras

In vertebrate embryos, the optic cups develop as lateral outgrowths of the diencephalon, the rudiments of which are first recognised at the antero-lateral margins of the neural plate. Evidence accumulating from extensive studies with vital dyes^{1–4}, excision and transplantation experiments^{5–7} in amphibian embryos have shown that the cell boundaries of the different areas of the central nervous system are already fixed at the early neurula stage when the presumptive retinal cells are located in the antero-medial border of the neural plate. It has remained unresolved whether the two retinal primordia and the layers thereof are determined in a single or separate group of cells. It seems to us that the problem is one of cell lineage and can be analysed with genetic mosaics. The retinal pigment epithelium (RPE) is derived from the outer layer of the embryonic optic cup. In chimaeric mice, produced by aggregating morulae from albino and pigmented strains, the presence of two types of cells in the RPE has been reported^{8–10}. Deol and Whitten¹¹ first noted that RPE of the two eyes of such chimaeras showed similar proportions of cells. It was recently observed that there is a close correlation in occurrence of chimaerism in the RPE and in the neural retina and between the two eyes of the individuals^{12–17}. Here we present quantitative data on the relative proportion and spatial distribution of cells from the two donor genotypes in the RPE and compare them with the relative proportion of cells in the neighbouring melanocyte population of the choroidal layer of the eye. The results show that there is a marked similarity in the proportion of the cells of the RPE from the two eyes of the individual chimaeras suggesting a lineage relationship between the two retinal primordia.

Chimaeric mice were produced by aggregation of morulae from albino BALB/c and pigmented C3H strains¹². The eyes were removed when the animals were 20–26 days old and 8 μ m thick serial sections were prepared. Out of 67 individuals obtained, 43 had both albino and pigmented cells in their RPE. Of these only 2 animals showed unilateral chimaerism, whereas in the rest the RPE of both eyes were chimaeric. Eyes from 20 animals, including the two showing unilateral chimaerism and representing a broad spectrum of distribution, were used in this study. The RPE from every third section was first drawn with a camera lucida and the stretches of albino and pigmented cells were marked. Each drawing was scanned in an x–y plotter attached to a computer. The program was designed to straighten the curved profiles of the RPE, to measure the linear lengths and calculate the relative proportions of the two components, and to map the spatial distribution of the cells in a two-dimensional reconstruction by aligning the linear outputs of the successive sections. In contrast to the RPE which is a single layer of cells, the choroidal melanocytes are located in a thick multicellular layer. For quantitative assessment every tenth section was used and the relative proportion of the albino cells in alternate stretches of the sections in the microscopic field were scored as 100% or 0% if completely albino or uniformly pigmented, 50% when the two kinds of cells appeared equal in proportion, 75% or 25% when the albino cells appeared to be relatively more or less than the pigmented cells; the mean value from all the scorings in each eye, numbering 150–180, was taken.

When the relative proportions of the albino cells in the RPE of the left and the right eyes from each chimaera are

listed in the decreasing order (Table 1) it is seen that the two individuals with unilateral chimaerism represent the two extremes with 99.6% and 0.4% albino cells respectively. Among the 18 individuals with chimaeric distribution in both eyes, the relative proportions of the albino cells vary from 99.6% to 5%; but show a high degree of correlation between the two eyes in each of these chimaeras. In the melanocyte population of the choroidal layer unilateral chimaerism is seen in 6 individuals. In 4 other animals chimaeric distribution is seen in the RPE but the choroidal melanocytes of both eyes are non-chimaeric. In animals showing chimaeric distribution in both layers the relative proportion of the cells in the choroid often differs considerably from the relative proportion in the RPE of the same eye. The difference in the relative proportion of the cells in the choroid layer of the two eyes of the individual chimaeras is also considerably more than that of the RPE.

The spatial pattern of cell distribution in the RPE of the two eyes from 10 out of 20 chimaeras analysed is shown in Fig. 1. Although examination of serial sections reveals that stretches of albino and pigmented cells may vary from a single cell to one of many cells, in the reconstructed map of the entire RPE, it is clearly seen that the two kinds of cell are not randomly scattered though considerable intermingling has occurred. Areas of varying sizes with predominantly one type of cell could be seen in all cases and often such areas appeared as radial sectors stretching from the optic nervehead to the periphery. The number and dimension of such sectors varied among the individuals but in a few cases 10 alternate sectors with predominantly albino and pigmented cells, as reported previously¹⁰, could be recognised (Fig. 1. 48 left eye, 45 right eye).

A similarity between the RPE of the two eyes could conceivably result from random assortment if the two types of cells were uniformly mixed throughout the chimaeric embryo. Observations on chimaeric blastocysts of mice, in which cells of one component were labelled with H³-thymidine (ref. 13) and in early post-implantation embryos of mouse $\longleftrightarrow$ rat chimaeras, in which the cells could be

Table 1 Relative proportions of albino cells (%) in the retinal pigment epithelium (RPE) and in the melanocyte population of the choroidal layer (CH) from the left and the right eyes of albino–pigmented chimaeric mice

Animal no.	Left eye		Right eye	
	RPE	CH	RPE	CH
23	99.6	100	100	100
2	99.6	100	99.3	88.1
16	98.8	100	98.2	100
8	98.5	84.7	98.1	100
10	97.2	88.2	90.8	100
49	96.4	100	95.0	100
56	95.6	98.7	95.7	93.8
29	95.5	100	95.2	97.8
63	93.2	94.9	89.6	100
48	85.6	99.1	77.3	88.9
69	82.7	82.2	72.0	94.3
9	78.8	87.5	72.5	91.6
45	75.9	74.0	77.0	67.8
18	66.9	77.2	61.6	60.8
3	58.8	70.1	72.0	83.9
1	49.8	76.1	38.8	58.1
54	32.5	30.1	37.0	63.3
20	13.3	24.5	12.1	56.0
44	5.0	14.9	7.5	0
14	0.4	0	0	0

The relationship between the percentage of albino cells in the RPE of the left and the right eye can be expressed by the following linear regression equation: $y = 0.8 + x$ (y , right eye; x , left eye) and the coefficient of determination (r^2) is 0.97. The difference between the RPE of the two eyes did not reach statistical significance (t dependent; $t = 1.4003$ d.f. 19). The absolute difference in the relative proportion of albino cells between the choroidal layer of the left and the right eyes is greater than that of the RPE (t dependent; $t = 3.226$ d.f. 19 $P < 0.005$ one-tailed).

identified by immunofluorescence¹⁴, suggest that the cells from the two donor components are not intimately mixed but remain in coherent clusters. Transplantation of presumptive retinal cells in early stage *Axolotl* embryo has been shown to result in growth of the cells derived from the graft within well defined radial sectors of the RPE¹⁵. Although more random distribution of the cells in the RPE of 12½-day-old chimaeric mouse embryos in comparison to that of the adult RPE has been reported¹⁶, there is no indication that at 12.5 d or at earlier stages of development

cell mixture was uniform throughout the RPE. Localised distribution of pigmented cells in the RPE of animals with more than 95% albino cells, as observed in this study, makes it unlikely that cell mixture could have been uniform enough to ensure equal division by random process.

The bilaterality of cell distribution in the RPE of chimaeric mice, more particularly in the individuals showing preponderance of one of the cell types, suggests an orderly mechanism of cell deployment. It is concluded that the cells giving rise to the two optic cups are derived from

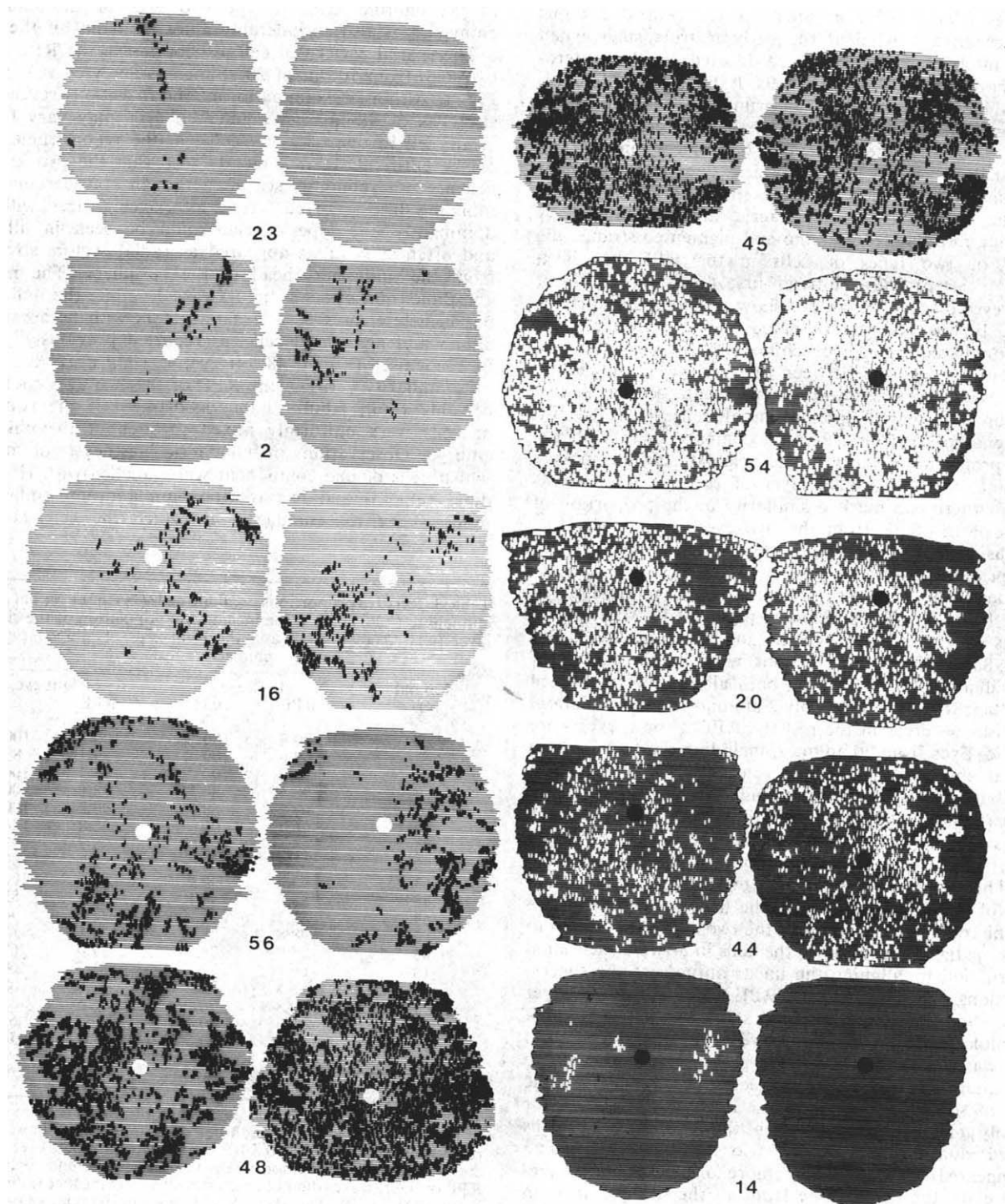


Fig. 1 Spatial distribution of albino (white) and pigmented (black) cells in the retinal pigment epithelium of the left and the right eyes from a series of chimaeric mice. The numbers indicate individuals listed in Table 1. The circle near the middle of each figure represent the optic nerve head.

common precursor cells, the lineage of which is so programmed that the daughter cells are segregated into two discrete lateral groups. It seems likely that such a mechanism may operate in the development of bilaterality of the central nervous system as a whole.

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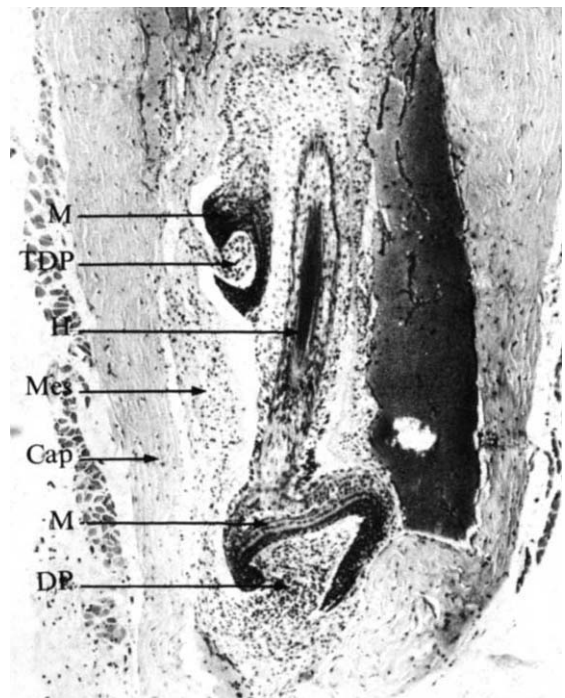


Fig. 1 A longitudinal section through a C₃H mouse (female) follicle given 2,500 rad of X irradiation. There is no trace of the matrix or other epithelial elements in the hair canal. ($\times 100$.) DP, dermal papilla; BS, blood sinus; Cap, capsule.

Inductive capacity of irradiated dermal papillae

MAMMALIAN hair follicles are specialised skin appendages which produce a series of hairs, one after another, in a regular cyclical fashion. The hair itself is composed of dead epithelial cells containing large amounts of keratin. During growth of the hair the epithelial cells at the bottom of the follicle surround the dermal papilla and divide rapidly. The dermal papilla is derived from mesenchyme and is composed of non-dividing cells. The importance of the dermal papilla in maintenance and control of hair cycles has been assumed for many years but little direct evidence of its function has been demonstrated. Geary¹ caused permanent removal of rat body hair by applying 1,720 rad of X irradiation. He believed that he had caused destruction of the dermal papilla which in turn resulted in permanent epilation. We report here a study on the effect of heavy doses of irradiation on the dermal papilla and epithelial elements of the hair follicle. We have found that high doses of irradiation, which cause permanent epilation, destroy the epithelium but leave the dermal papilla anatomically and functionally intact so that even on transplantation it can induce a series of new hairs.

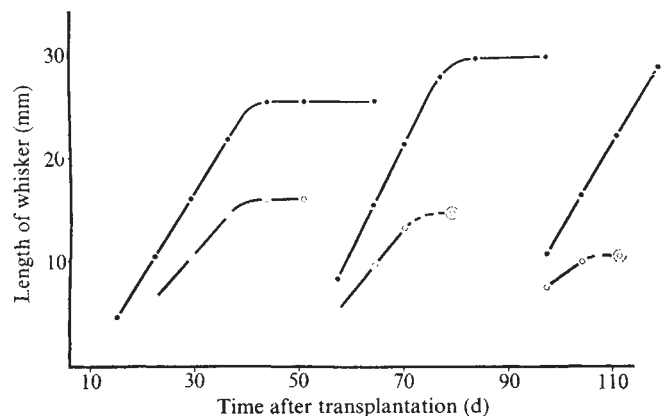
The mystacial vibrissal follicles of rats and mice form a remarkably constant pattern. Each follicle regularly produces a series of vibrissae with constant characteristics. Their lengths can be easily measured in the live animal by the capillary tube method^{2,3}. The four largest vibrissal follicles, designated by Danforth⁴ as E, F, G and H, form a vertical column in the most posterior position and the 'a' column of five slightly smaller vibrissal follicles are found immediately anterior.

A single dose of X ray was applied locally to the vibrissal follicles in young mice. Permanent epilation in the E, F, G and H column of follicles took place after a dose of 2,500 rad (ref. 5). Five months later these follicles were examined histologically and were found to have an isolated dermal

papilla at the bottom of the follicle. This dermal papilla was rounded in shape and packed with cells. No epidermal cells were seen (Fig. 1). Permanent epilation was therefore considered to be a result of complete destruction of the epidermal constituents of the follicle while leaving the dermal papilla histologically intact.

To determine whether the dermal papilla in a heavily irradiated follicle is physiologically functional, it would be necessary to bring the irradiated dermal papilla into contact with normal follicular epithelium. To achieve this E, F, G and H vibrissal follicles in Wistar-derived rats were irradiated with 5,000 rad of X irradiation and on the next

Fig. 2 Growth curves of the two rat whiskers observed growing from Ia follicle after transplanting a heavily irradiated dermal papilla into its hair canal. The normal was a few mm shorter in the first cycle probably because of damage during transplantation. The whisker induced by the transplant became shorter in each successive cycle. ●, Normal whisker; ○, whisker induced by the transplanted irradiated dermal papilla; ⊙, whisker absent.



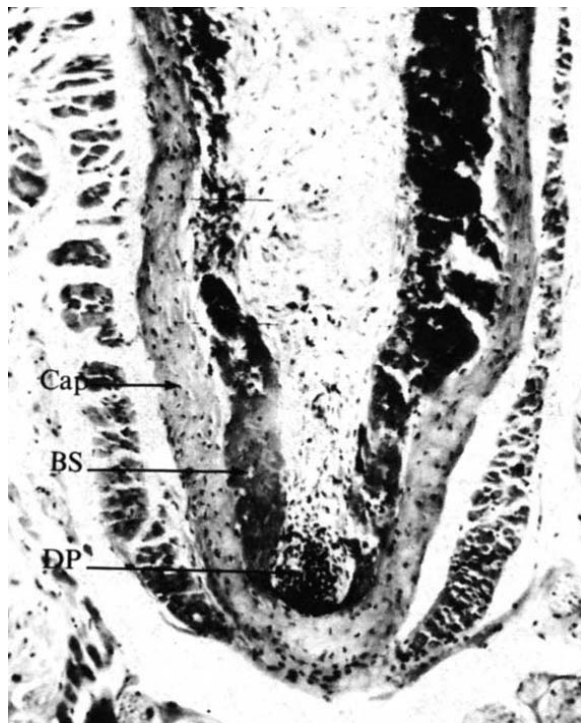


Fig. 3 The bottom end of an F follicle which received two dermal papillae irradiated with 5,000 rad. The vibrissae are inside the hair canal. The superior transplanted dermal papilla has made connection with the mesenchymal blood plexus. The second transplanted dermal papilla has distorted the normal at the bottom of the follicle and is not clearly shown in this section. ($\times 95$.) Abbreviations as in Fig. 1; also M, matrix; Mes, mesenchymal sheath; H, hair induced by the host dermal papilla; TDP, highly irradiated, transplanted dermal papilla.

day the animals were killed and their dermal papillae were excised and transplanted into normal vibrissal follicles in normal rats. Three to four weeks later an extra whisker was observed growing alongside the normal vibrissa of the host follicle in approximately one third of the follicles. The whiskers induced by the irradiated dermal papillae were observed for two to three hair cycles before the rat either died or was killed. The induced vibrissae were shorter than the normal in length and had a slower rate of growth (Fig. 2).

Histologically, the induced hair bulb looked normal in most respects except for its situation (Fig. 3). We concluded that new hairs were induced by the transplanted dermal papillae wherever they were placed inside the hair follicle even if it was in the blood sinus. The length and size of the induced hair was dependent on the size of the transplanted dermal papilla. The whiskers produced by the irradiated and transplanted dermal papillae became shorter in successive cycles. They were more than 30% shorter at the end of three successive cycles.

Thus if heavily irradiated, permanently epilated follicles are left *in situ* the epithelium is destroyed but the dermal papilla remains as a recognisable but rounded mass of apparently viable cells. Transplantation of heavily irradiated dermal papillae into normal vibrissal follicles, however, induced additional new hairs which were observed for up to three successive cycles.

These findings show that a massive dose of X irradiation eliminates the epithelial elements of the growing hair, but does not abolish the inductive capacity of the dermal papilla. The failure of these dermal cells to display damage during at least 5 months observation following irradiation

probably occurs because they do not divide; an analogous situation is found in the resistance to radiation of adult mammalian neurones⁶.

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Vesicular disruption of myelin simulated by exposure of nerve to calcium ionophore

EXPERIMENTAL allergic encephalomyelitis (EAE) and experimental allergic neuritis (EAN) are autoimmune demyelinating conditions brought about by sensitisation to central and peripheral myelin extracts, respectively. The lesions are the result of a delayed hypersensitivity-type reaction which can be passively transferred by lymphoid cells but not by sera^{1–4}. Demyelination occurs in areas of cellular infiltrate. This process is mediated in part by mononuclear cells which invade and phagocytise the target myelin tissue. A vesicular transformation of myelin sheaths is known to occur in close proximity to these invading mononuclear cells^{5–7}. The nature of the myelin vesiculation is unclear; however, these changes have been emphasised as a characteristic accompaniment of autoimmune demyelination in experimental animals and in human peripheral nerve disease⁸. The present study reports the occurrence of the same type of myelin alteration in excised rat peripheral nerve fibres when incubated under conditions which promote a selective influx of calcium ions into the tissues.

Multiple 15-mm segments from the same excised and desheathed rat sciatic nerves were simultaneously incubated in oxygenated Ringer's solutions with and without the addition of A23187, 10 mg l⁻¹, and in calcium-free Ringer's solution containing ionophore and EGTA, 1 mmol l⁻¹, to chelate endogenous calcium. After 30-, 60- and 120-min incubations at 37 °C, the tissues were fixed by immersion and processed for electron microscopic examination as already described⁹.

A vesicular disruption of myelin appeared as a distinctive alteration of nerve fibres incubated in the presence of both calcium and A23187. These changes were characterised by progressive vesicular transformation of myelin occurring along the surfaces of myelin sheaths, leading to increasing accumulations of a vesicular or foamy membranous meshwork adjacent to the remnants of original myelin sheaths. After 30 min, early vesicular changes could be seen as multiple vesicular eruptions along the inner and outer surfaces of compact myelin sheaths (Fig. 1). Progression of vesicular alterations was evidenced after 60 and 120 min by massive accumulations of a foamy material in myelinated fibres (Fig. 2). Vesicular alterations occurred predominantly in nerve fibres situated around the periphery of the nerve fascicles immediately beneath a thin residual perineurial investment (Fig. 3). The location of these fibres rendered them most susceptible to the effects of the incubation media as well as the fixative solutions. The penetration of lipophilic ionophore may also have been limited by the high lipid content of the nerve tissue.

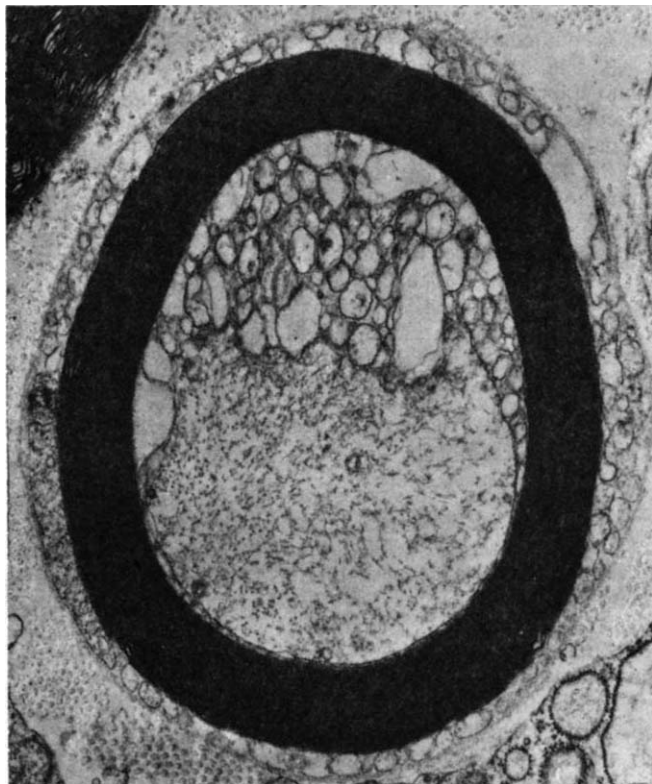
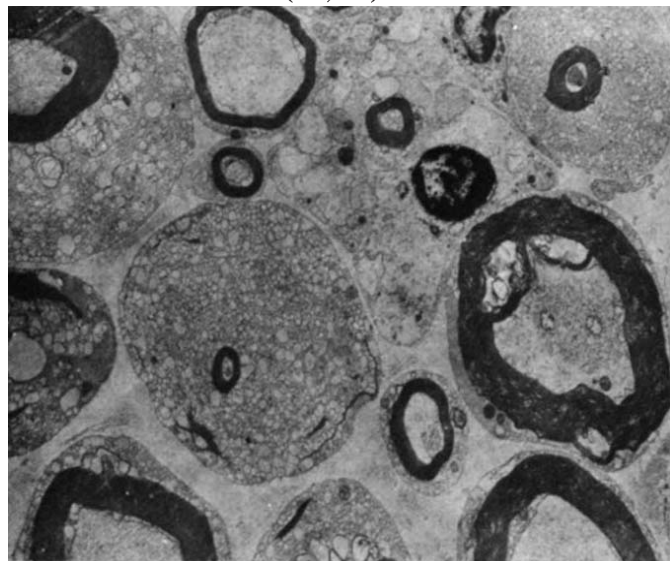


Fig. 1 Cross section of myelinated rat peripheral nerve fibre showing early vesicular disruption of myelin after 30-min exposure to A23187 ($10 \mu\text{g l}^{-1}$) in Ringer's solution containing CaCl_2 (4 mmol l^{-1}). Arrays of vesicular profiles occur along the inner and outer surfaces of the otherwise intact lamellar myelin sheath. ($\times 14,000$.)

A vesicular disruption of myelin was not seen in control nerve segments incubated in the absence of either ionophore or calcium. The vesicular type of myelin alteration was readily differentiated from preparative myelin artefacts which were scattered through the nerve fascicles and occurred with limited but equal frequency in experimental and control tissues. These myelin artefacts were characterised by concentric loosening of myelin lamellae or by

Fig. 2 Cross section of nerve fascicle showing several fibres with more advanced states of myelin vesiculation following 120-min incubation in Ringer's solution containing A23187. A vesicular or foamy network has accumulated in Schwann cell cytoplasm adjacent to the residua of compact myelin sheaths. ($\times 3,000$.)



focal distortions or extrusions in the laminated cylindrical form of the myelin sheath.

Incubations were conducted between pH 7.2 and 7.4, and visually monitored by inclusion of phenol red ($1:10,000$) in the media. Under these conditions, A23187 has selective high affinities for calcium and magnesium^{10,11}. Both experimental and control incubations contained MgCl_2 (1 mmol l^{-1}), but only the experimental incubation contained both ionophore and calcium, a condition associated with the influx of calcium into tissues. The ability of A23187 to translocate calcium across cell membranes is well known and is believed to be due to a physical envelopment of calcium by ionophore, thereby rendering calcium lipid soluble and membrane permeable¹². The direction of ionophore-mediated transmembranous movement of calcium is down the calcium concentration gradient. In calcium-free media containing EGTA, the calcium gradient is reversed. Incubation of nerve under these conditions caused separation of axolemmae from internal Schwann cell membranes of myelinated fibres, morphological features indicative of reduced tissue calcium observed following calcium deprivational conditions in tissue cultured peripheral nerve¹³. The absence of myelin vesiculation under these conditions suggests the existence of a subthreshold calcium level, insufficient to induce myelin vesiculation.

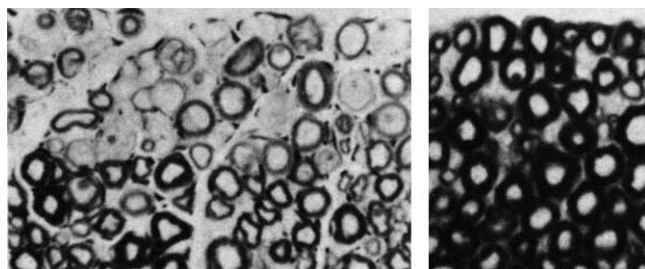


Fig. 3 Comparison of myelinated fibres along lateral edge of experimental (left) and control (right) nerve segments incubated for 120 min in Ringer's solutions with and without A23187. Superficial myelinated fibres in the experimental nerve segment reveal dissolution of compact myelin manifested by a loss in density of circular profiles, which represent cross sections of compact myelin sheaths. Additional control incubations in media containing ionophore but lacking calcium showed uniform preservation of compact myelin sheaths. Thin section of epoxy-embedded tissue stained with toluidine blue. ($\times 450$.)

These results imply the existence of calcium-reactive sites within the myelin sheath which induce myelin vesiculation upon their exposure to calcium. These calcium-reactive sites seem to be located within the myelin lamellae in such a manner as to render them inaccessible to calcium in the extracellular spaces in physiological conditions or to calcium present in the intraperiod space during incubations in hypotonic Ringer's solution¹⁴. Their relationship to calcium-binding sites of myelin¹⁵ is unknown. Calcium-binding sites of plasma membranes may be exposed by detergent pretreatment¹⁶. The vesiculation of myelin which occurs in nerves exposed to lysophosphatidyl choline¹⁷ could exemplify a detergent-induced exposure of calcium-reactive sites in the myelin sheath.

Myelin vesiculation which occurs in pathological conditions may also represent the manifestations of calcium interaction with components of the myelin sheath. Exposure of calcium-reactive sites could well occur during the degradation of myelin sheaths, resulting in occasional focal vesicular alterations reported or depicted in these circumstances¹⁸⁻²². The myelin vesiculation of cell-mediated immunological demyelination, however, is distinguished by its prominence and occurrence along the surfaces of intact myelin lamellae in the vicinity of infiltrating mononuclear

cells⁷⁻⁸. The distribution of change in close proximity to these cells is strongly indicative of their complicity in the causation of myelin vesiculation. Numerous humoral factors have been identified as mediators of cellular immunological reactions²³ although the mechanisms underlying their activities have often remained obscure. A humoral mediator of myelin vesiculation would have particular interest in that its mechanism of action may well be operative by promoting calcium penetration into myelin membranes. A calcium influx is well recognised as a trigger for many biological phenomena. Accordingly, a humoral factor with the ability to increase calcium permeability of membranes could serve as a powerful means of effecting immunological reactions.

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Cancer-induced cytotoxicity of normal bone marrow cells

MOST investigations of the invasive process of cancer have involved microscopic studies of tumour spread after injection of cancer cells into experimental animals¹⁻⁴. Attempts to investigate tumour invasiveness using organ or parabolic cultures have met with limited success⁵⁻⁷. We have evaluated tumour-mediated cytotoxicity of normal rat marrow cells using suspension cultures containing Walker-256 carcinosarcoma cells and ⁵⁹Fe-labelled rat bone marrow cells. This mixed cell culture system seems to provide a promising approach to the further understanding of cancer invasiveness.

Interactions between cancer cells and normal cells were evaluated by a modified cytotoxicity assay⁸. Walker-256 carcinosarcoma cells (from Arthur D. Little Co. under the auspices of the NIH) were isolated from the ascites tumour which accumulated 7 d after intraperitoneal transplantation of 1×10^6 Walker-256 cells to adult Wistar rats. Tumour cells were separated from erythrocytes by centrifugation (400g) on a sterile gradient of a sucrose polymer and diatrizoate salts (LSM Solution, SG 1.077-1.080, Litton). Tumour cells, located at the interface, were collected, washed twice and suspended in nutrient medium. More than 98% of the isolated cells were identified morphologically as tumour cells.

Rat marrow target cells were collected and prepared for suspension culture as before⁹. Bone marrow cells (1×10^7 per ml) were labelled with $2 \mu\text{Ci } ^{59}\text{Fe}$ per ml (ferrous citrate, Mallinkrodt) for 4 h at 37 °C. (After a 4-h incubation of rat marrow cells with

⁵⁹Fe-transferrin, Storing and Fatih reported that cytoplasmic ⁵⁹Fe accounted for approximately 78% and stromal ⁵⁹Fe for 22% of all ⁵⁹Fe incorporated¹⁰. Cytoplasmic ⁵⁹Fe consisted of haemoglobin iron (65%), ferritin iron 20% and an unidentified low molecular weight iron (15%).) The cells were washed four times to eliminate unincorporated label, suspended in nutrient medium (70% NCTC-135, 30% heat-inactivated foetal calf serum, Grand Island Biological, with penicillin and streptomycin) and added to various concentrations of Walker-256 cancer cells. The cell suspensions were mixed thoroughly by inversion and incubated in 10×75 -mm friction cap tubes (Falcon) in 5% CO₂ and 95% air for 18 h at 37 °C. The mixed cell suspensions were then centrifuged at 400g for 10 min (4 °C), and the radioactivity in the supernatant and cell pellet was measured. Cytotoxicity is expressed as a function of the release index (RI)

$$\text{RI} = \frac{\text{radioactivity released due to cell death (supernate)} \times 100}{\text{total radioactivity (supernatant and cell pellet)}}$$

Because most of the ⁵⁹Fe is taken up by haemoglobin-producing nucleated marrow cells (erythroblasts)¹¹, this technique measures principally the release of haemoglobin and non-haemoglobin iron-containing molecules from erythroblasts, and has been termed erythroblast cytotoxicity assay¹². As in lymphocyte cytotoxicity studies¹³, the baseline RI of marrow cells varied between experiments, making control cultures essential (⁵⁹Fe-labelled marrow cells alone) with each experiment. Significant differences between triplicate cultures of marrow cells alone and marrow cells cultured with Walker-256 cells were determined by Student's *t* test¹⁴.

The ability of Walker-256 cancer cells to initiate marrow cell cytotoxicity is typified by the data in Table 1. Similar results were

Table 1 Marrow cytotoxicity: Trypan blue exclusion (viability) and RI

Ratio tumour cells to marrow target cells	RI (% $\pm$ s.e.m.)	Marrow viability (%)
0:1	18.5 $\pm$ 1.3	90
0.13:1	27.5 $\pm$ 1.0*	73
0.25:1	32.4 $\pm$ 1.0*	77
1:1	49.3 $\pm$ 1.2*	59
10:1	60.6 $\pm$ 1.4*	—
1 (alcohol-treated):1	17.9 $\pm$ 0.8	88
Tumour cell sap: 1 (from 1×10^8 cells)	18.3 $\pm$ 0.7	87
Ascitic fluid supernate:1	18.1 $\pm$ 0.8	76
1 Mouse spleen:1	21.0 $\pm$ 0.4	87
1 Rat spleen:1	18.9 $\pm$ 0.6	89

RI, release of ⁵⁹Fe from labelled rat bone marrow cells incubated for 18 h with viable Walker-256 carcinosarcoma cells, alcohol-treated Walker-256 cells, cell-free tumour ascitic fluid, or normal spleen cells.

**P* < 0.01, significantly different from control cultures containing ⁵⁹Fe-labelled rat marrow cells alone (0 tumour cells to 1 marrow cell).

obtained in nine other experiments. The spontaneous RI for marrow erythroblasts cultured alone was $18.5 \pm 1.3\%$ (s.e.m.). The increasing cytotoxicity of marrow cells cultured with Walker-256 cells was proportional to the ratio of tumour cells to marrow cells. In cultures containing equal numbers (1×10^7 cells) of Walker-256 and marrow cells the RI was $49.3 \pm 1.2\%$. Marrow cell viability, as determined by Trypan blue exclusion, was inversely proportional to the number of tumour cells in culture. ⁵¹Cr-labelling of rat marrow cells also resulted in significant, but less tumour-mediated marrow cytotoxicity.

Tumour-mediated cytotoxicity of erythroblasts required intact tumour cells. No cytotoxicity was noted with alcohol-treated Walker-256 cells or the supernatant from the tumour ascitic fluid (Table 1). The cell sap of sonicated Walker-256 cells (100,000g for 90 min) also had no effect on erythroblast cytotoxicity. When the effect of temperature on marrow RI was evaluated, no tumour-induced marrow cytotoxicity was observed in 24-h cell cultures maintained at 4 °C and was minimal at 25 °C. Maximum marrow cytotoxicity was observed at 37 °C.

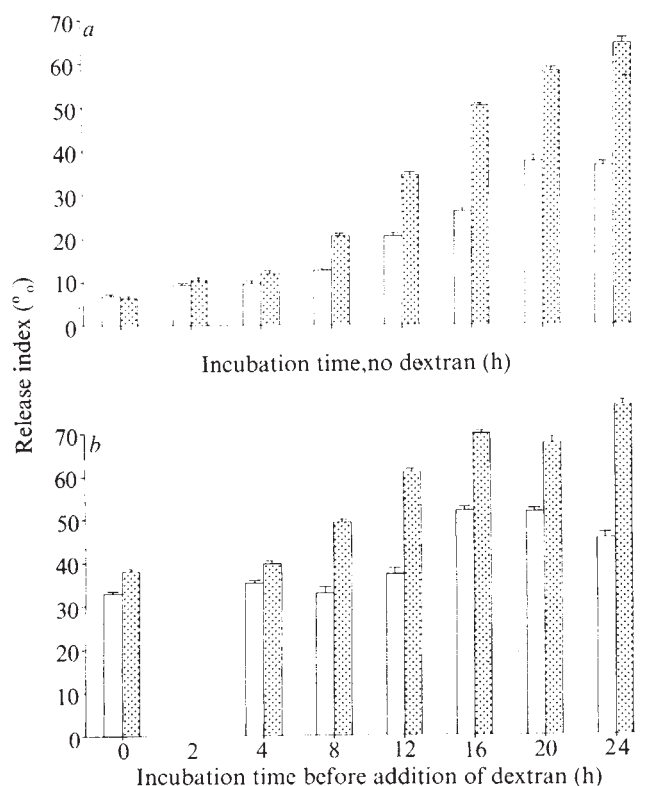
To evaluate the ability of non-malignant cells to mediate marrow cytotoxicity, ⁵⁹Fe-labelled normal rat marrow cells were

cultured with an equal number of normal mouse or rat spleen cells. Spleen cells did not significantly increase rat marrow erythroblast cytolysis (Table 1).

The progression of tumour-induced marrow cytolysis was evaluated by incubating tumour and marrow cells together and measuring RI at 0, 2, 4, 8, 12, 16, 20 and 24 h. As Fig. 1a shows, no significant increase in the release of ^{59}Fe from marrow cells was evident before 4 h, but marrow cytolysis increased during the next 12 h.

The requirement for intercellular contact between tumour cells and marrow cells during cytolysis was evaluated by a modification of Martz's method⁸. Equal numbers of Walker-256 cancer cells and ^{59}Fe -labelled marrow cells were incubated for 24 h in suspension cultures as described above. At specified intervals, however, 3 volumes of 10% high molecular weight Dextran (average 500,000: T-500, Pharmacia) in complete culture medium was added to each culture and mixed. Incubation was then completed and RI was measured. As Fig. 1 shows, erythroblast cytolysis increased as the duration of cell contact (before addition of Dextran) increased. When cell interaction was minimised by addition of high molecular weight Dextran at the initiation of culture (0 h), tumour-mediated marrow cytolysis was minimal.

Fig. 1 Cytolysis of ^{59}Fe -labelled marrow cells cultured alone (senescent lysis, clear bars) or with an equal number of tumour cells (senescent lysis and tumour-mediated lysis, shaded bars). *a*, Temporal progression of marrow cytolysis. Suspension cultures of marrow cells or marrow and tumour cells in nutrient medium were initiated at $t=0$ and terminated after 0, 2, 4, 8, 12, 16, 20 or 24 h of incubation. The cultures were centrifuged and the RI (mean $\pm$ s.e.m.) of triplicate cultures was calculated. *b*, Requirement for continuous marrow-tumour cell interaction in order to obtain maximal tumour-mediated marrow cytolysis. Suspension cultures of marrow cells or marrow and tumour cells were initiated at $t=0$ and after 0, 4, 8, 12, 16, 20, or 24 h of culture, 3 volumes of 10% Dextran in complete medium were added with vigorous mixing. Cultures were re-incubated at 37°C to give a total of 24 h of incubation. Cultures were then terminated and the RI of triplicate cultures was calculated. Minimal tumour mediated marrow cytolysis was observed when Dextran was added within 4 h of the initiation of culture.



Walker-256 cancer-mediated cytolysis of ^{59}Fe -labelled rat erythrocytes (labelled *in vivo* by intravenous injection of ^{59}Fe into rats 3 d before use of erythrocytes) was also demonstrated. The spontaneous release (RI) of ^{59}Fe from rat erythrocytes cultured alone was 1.5% in 20 h. The RI of rat erythrocytes cultured with Walker-256 cells (1:1 ratio) was 37.7%.

To determine whether the cytolytic interaction of Walker-256 tumour cells and erythrocytes was independent of phagocytosis, the phagocytic ability of Walker-256 tumour cells was evaluated by the method of Walker and Demus¹⁵. Tumour cells, collected and prepared as described above, were incubated for various times with erythrocytes labelled *in vitro* with ^{51}Cr . At the end of incubation the cells were centrifuged and the radioactivity in the supernatant (S_1) was determined. The cell pellet containing tumour cells, phagocytised ^{51}Cr -labelled erythrocytes and intact ^{51}Cr -labelled erythrocytes was suspended in distilled water and left at room temperature for 15 min. By this technique the engulfed erythrocytes would be protected from hypotonic lysis and ^{51}Cr would remain with the pellet. The cells were then centrifuged and the radioactivity in the supernatant (S_2) and the pellet (P) was determined.

The phagocytosis index (%)

$$= \frac{\text{c.p.m. in pellet (P) after water lysis}}{\text{total c.p.m. (S}_1 + \text{S}_2 + \text{P)}} \times 100$$

was calculated. The phagocytosis of labelled erythrocytes by tumour cells was compared with the value obtained when ^{51}Cr -labelled erythrocytes, cultured without tumour, were subjected to hypotonic lysis, and the pellet-associated radioactivity was determined as described above. The phagocytosis index for erythrocytes cultured alone was $10.9 \pm 2.2\%$ and for erythrocytes cultured with tumour was $12.6 \pm 0.5\%$. These values are not significantly different from each other ($P > 0.1$). Thus, in contrast to the murine reticulum cell sarcoma described by Ralph and Nakoing¹⁶, Walker-256 breast carcinosarcoma is cytolytic both to nucleated and non-nucleated erythroid cells, but has no phagocytic capability in the system described above. Marrow-tumour cell cultures were also examined by electron microscopy, revealing no evidence of tumour phagocytosis of marrow cells.

Our findings indicate that Walker-256 carcinoma cells mediate the cytolysis of rat erythroblasts and erythrocytes in suspension cultures by a cell-cell interaction which is temperature-dependent and independent of phagocytosis. This cytolytic effect requires intact, viable Walker-256 cancer cells and, in contrast to lymphocyte-mediated cytotoxicity, will occur with a low effector cell-to-target cell ratio.

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H-2^a- associated alloantigen expressed by several transplacentally-induced lung tumours of C3Hf mice

CELL surface changes accompanying malignancy may result in the expression by the tumour cells of antigens recognisable by the host's immune system. It is interesting to know whether nascent tumours express common tumour-associated antigens and what, if any, is the relationship of these antigens to histocompatibility alloantigens. We have addressed these questions by testing a series of early passage, transplacentally induced murine lung tumours for a cross-reactive histocompatibility locus-coded alloantigen. A common transplantation alloantigen was readily detected on six of 15 lung tumours tested.

According to the concept of immune surveillance, a major function of the immune system is the detection and elimination of nascent autochthonous tumours¹⁻³. The cytotoxic T-cell-mediated immune response evoked in mice against allogeneic cells is directed primarily against cell surface components coded for by the K and D regions of the H-2 major histocompatibility complex (MHC) (ref. 4). The specificity of T-cell-mediated immunity against chemically modified^{5,6} or virally infected⁷⁻¹¹

occur during repeated passage of the tumour. Studies of the specificity of the MHC-coded antigens expressed by long term transplanted tumours cannot, therefore, be unequivocally extrapolated to the MHC-coded antigens expressed by nascent tumours. Therefore, we have studied 14 additional transplacentally induced lung tumours of C3Hf mice after either the 1st or 2nd *in vivo* passage for the expression of the strain-A-associated alloantigen detected on the 85 lung tumour.

Lung tumours were induced by a single transplacental administration of 1-ethyl-1-nitrosourea (ENU) to pregnant C3Hf mice (0.5 μ mol per g body weight). The mice were injected between days 13 and 16 of gestation. Progeny mice were killed at approximately 1 yr of age and lung tumours greater than 5 mm in diameter were transplanted as fragments into syngeneic C3Hf mice. Fourteen tumours grew on 1st passage. Some of the tumours were retransplanted into a second group of syngeneic recipients. Tumour tissue from either the 1st or 2nd *in vivo* passage was collected and cut into approximately 1-mm square fragments. These fragments were stored in a 10% dimethyl sulphoxide solution under liquid nitrogen. Groups of C3Hf and (C3Hf $\times$ A)_F₁ hybrid mice were inoculated intradermally with a single fragment of tumour as described previously^{15,16}. Tumours which grew were excised from the tumour-inoculated mice. Control C3Hf mice were inoculated with a lung tissue fragment from either strain B10.A (H-2^a) or B-10 (H-2^b) mice. Immunised mice were X-irradiated (400 R) and challenged

Table 1 Ability of six transplacentally-induced lung tumours of C3Hf mice to induce radioresistant immunity in C3Hf, but not in (C3Hf $\times$ A)_F₁ mice, against the 85 tumour

Pre-immunisation	Tumour growth in X-irradiated mice			
	Tumour incidence	C3Hf mice Mean tumour diameter $\pm$ s.e. (mm)	Tumour incidence	(C3Hf $\times$ A) _F ₁ mice Mean tumour diameter $\pm$ s.e. (mm)
Nil	10/10	10.8 $\pm$ 1.1	8/8	15.1 $\pm$ 1.0
85	1/9	0.3 $\pm$ 0.3*	8/8	12.0 $\pm$ 1.6
38	1/10	0.4 $\pm$ 0.3*	8/8	12.3 $\pm$ 1.4
292 F-3	3/10	3.9 $\pm$ 1.8*	9/9	17.3 $\pm$ 1.0
294 F-2	1/10	0.4 $\pm$ 0.4*	9/9	16.4 $\pm$ 0.8
320 M-1	2/9	3.3 $\pm$ 1.3*	10/10	15.0 $\pm$ 0.8
321 M-3	7/10	4.9 $\pm$ 1.1*	9/9	14.0 $\pm$ 0.6
Other tumours (9 total)	88/89	10.3 10.8 11.3 12.3 12.6 12.7 13.2 14.1 15.7	NT	NT
B10 lung	8/8	12.5 $\pm$ 0.8	NT	NT
B10.A lung	0/8	0.0 $\pm$ 0.0	NT	NT

Palpable tumours were present at 1 week after tumour inoculation in all (C3Hf $\times$ A)_F₁ recipients and in all but one C3Hf recipient which received tissue from the nine non-cross-reacting lung tumours. As indicated by tumour incidence, most of the C3Hf mice pre-immunised with tissue from the six cross-reacting lung tumours failed to develop tumours during a 2-month observation period. The mean diameters ($\pm$ s.e.) recorded here were those obtained 4 weeks after tumour inoculation in the C3Hf mice and 3 weeks after tumour inoculation in the (C3Hf $\times$ A)_F₁ mice. Results marked with an asterisk indicate a significant difference from tumour growth in that group compared with tumour growth in the group not pre-immunised ($P < 0.001$). The other values were not significantly different from the control group ($P > 0.5$). NT, not tested.

syngeneic cells is also determined by these MHC-coded components. Cytotoxicity mediated by T lymphocytes against certain long term transplanted murine leukaemias can be inhibited by antisera directed against the H-2 antigens expressed by the leukaemia cells^{12,13}. We have demonstrated that the tumour-associated transplantation antigen on a transplacentally induced lung tumour of a C3Hf/HeN mouse is similar or identical to an antigen coded for by the K region of the MHC of strain A mice. Thus radioresistant syngeneic immunity can be induced against this tumour, designated 85, by immunising C3Hf mice with normal tissues of strains A, B10.A and B10.A (2R), but not C3Hf, B10 or B10.A (5R) mice¹⁴. These studies suggest that depressed or altered components coded for by the MHC may provide the mechanism of recognition of nascent tumours by the cellular immune system. The MHC is subject to mutation¹⁵ and antigenic deviation between the MHC-coded antigens expressed by a long term transplanted tumour and by the normal tissues of the mouse strain of tumour origin might

with 10⁵ 85 tumour cells. Tumour growth was measured at weekly intervals. Preimmunisation with five of the 14 lung tumours induced radioresistant cross-reactive immunity against the 85 tumour in C3Hf but not in (C3Hf $\times$ A)_F₁ mice (Table 1). The linkage of the 85 tumour associated antigen to the MHC of H-2^a mice is shown by experiments which indicate that anti-85 immunity can be induced in C3Hf mice by preimmunisation with lung tissue of B10.A but not B10 mice (Table 1).

The results indicate that six out of 15 (40%) of the transplacentally induced lung tumours of C3Hf mice studied express a common cross-reacting transplantation antigen which is similar or identical to a normal tissue alloantigen of H-2^a mice. The finding of this common transplantation, MHC-coded, alloantigen is consistent with the important role attributed to modified or derepressed MHC products in the recognition of nascent autochthonous tumours^{3,12,13,16,17}. The frequency with which the H-2^a-associated antigen is expressed also suggests that a clearly definable alteration in genetic regulation or syn-

thesis of the MHC-coded components is associated with lung tumour development in C3Hf mice.

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Antibody-enhanced dengue virus infection in primate leukocytes

DENGUE viruses, types 1-4, are arthropod-borne flaviviruses which cause dengue shock syndrome (DSS) in humans possessing pre-infection antibody, passively acquired or derived from heterotypic infection¹. Although the immunopathological mechanism of DSS is not fully understood, experimental studies suggest that dengue virus production is immunologically regulated. Monkeys with monotypic immunity to dengue types 1, 3 or 4 viruses, when challenged with dengue 2, had significantly higher levels of circulating virus than did similarly infected susceptible animals². This may be attributable to the replication of virus in leukocytes. In infected monkeys, virus was frequently recovered from buffy coat cells and from lymphatic tissues³. The role of leukocytes in enhanced infection is further supported by the observation that dengue replicates readily in cultures of peripheral blood leukocytes (PBL) prepared from immune simian or human donors, but poorly or not at all in leukocytes from non-immune hosts⁴⁻⁶. Studies on the immunological specificity of this phenomenon have been hindered by the requirement either for expensive experimental hosts (monkeys) or for human donors with chronologically defined dengue infections. Here we describe the *in vitro* enhancement of dengue infection in PBL by antibody. This system provides a provisional model for DSS in young infants during primary dengue infections^{7,8}.

Dengue virus, type 2 (strain 16681) (D2V) propagated in a continuous rhesus kidney cell line, LLC-MK2, was used for all *in vitro* infections⁹. D2V seeds contained approximately 10^7 plaque-forming units (PFU) per ml.

Dengue antibodies were obtained from man, monkey, mouse and rabbit. Serum was collected from naturally infected humans, or raised in monkeys by bleeding animals one or more months after a single subcutaneous injection of one of four prototype tissue culture passaged dengue viruses⁹. This regularly induced infection with the production of antibodies whose serological characteristics have been described¹⁰. In mice, antibodies were raised by a

standard immunising schedule, using prototype mouse adapted dengue strains¹¹. Antibodies were obtained in ascitic fluids induced by sarcoma 180 cells¹². In rabbits, dengue antibodies were raised as described by DeMadrid and Porterfield¹³.

PBL were collected from humans, *Macaca mulatta*, *Macaca irus*, New Zealand rabbits, Duncan Hartley guinea pigs and several mouse strains. Sera from each leukocyte donor failed to neutralise dengue 1, 2, 3 and 4 viruses when measured at a 1:10 dilution in the LLC-MK2 assay⁹. PBL were separated from erythrocytes using dextran T-250 (refs 4-6) or by isopycnic centrifugation on Ficoll-Hypaque¹⁴. Viable cells were adjusted to either 1 or 1.5×10^6 mononuclear leukocytes per ml in RPMI-1640 supplemented with 10% heat-inactivated (56°C for 30 min) foetal calf serum (FCS), 20 mM HEPES, 0.02% NaHCO_3 , 2 mM glutamine and 200 U ml^{-1} penicillin and $200 \mu\text{g ml}^{-1}$ streptomycin. In some experiments heat-inactivated autologous or pooled normal monkey serum was substituted for FCS.

Dengue virus at a multiplicity of infection (MOI) of 0.1-0.01 was added to PBL suspensions containing various concentrations of dengue antiserum and to control cultures without dengue antiserum. Below a MOI of 0.001, infection did not usually occur. At first, virus, serum and PBL were incubated for 1 h at 37°C , cell suspensions were washed twice in 50 volumes of Hanks' BSS then resuspended in medium at 1×10^6 cells per ml. Cells were incubated in screw-capped glass or plastic vials at 37°C . Later, when

Fig. 1 Dengue 2 virus titres in cultures of normal rhesus peripheral blood leukocytes (PBL) in 20 paired comparisons, with (●) and without (○) anti-dengue 4. D2V was added to all cultures at MOI of 0.2-0.05. Virus was not removed by washing. To one half of cultures a single lot of anti-dengue 4 was added at a final dilution of 1:30. Control cultures were incubated with 1:30 autologous serum or pooled normal monkey serum. Mean plaque forming units were calculated from three 1-ounce LLC-MK2 bottles; averages of means are plotted $\pm$ s.e.m. The *t* test for paired data was performed using the square roots of mean plaque counts.

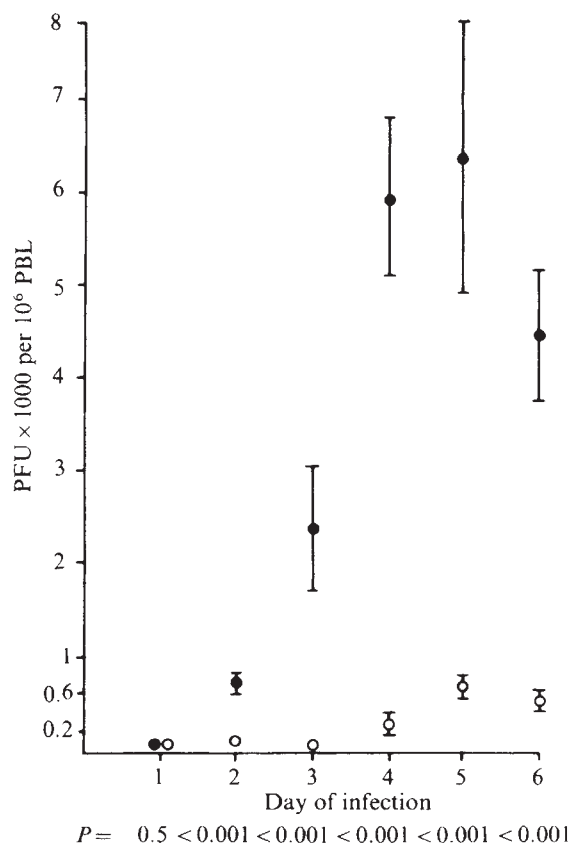


Table 1 Dengue 2 virus infection enhancement activity of serial 10-fold dilutions of anti-dengue sera raised in man, monkey, mouse and rabbit

Antibody specificity	Antibody donor species	PBL donor species	Reciprocal of titre		Against homologous virus* PRNT	PFU serum control at indicated dilution†			
			Against HI	D2V PRNT		10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
anti-D1	<i>M. mulatta</i>	rhesus	20	20	640	63	123	14	1
-D2	<i>M. mulatta</i>	rhesus	320	1,280	—	<1	<1	9	101
-D3	<i>M. mulatta</i>	rhesus	40	15	320	169	26	1.6	<1
-D3	rabbit‡	man	ND	320	160	6	13	10	1.4
-D4	<i>M. mulatta</i>	rhesus	40	12	640	16	28	1	1
-D4	man§	rhesus	10	<10	180	32	9	<1	<1
-D4	mouse	rhesus	40	18	320	12	29	12	1

*Serial four-fold dilutions of serum were tested against 5–40 (PFU) of homologous virus in a LLC-MK₂ plaque assay. Tests involved haemagglutination-inhibition (HI) or a plaque reduction neutralisation test (PRNT).

†Ratio between PFU on day 4 of rhesus PBL cultures inoculated with D2V (MOI = 0.1) plus anti-dengue (serum) divided by PFU in control cultures. Significant differences ($P < 0.05$) are shown in italics.

‡Serum described by De Madrid and Porterfield¹³. Tested in human PBL and virus in day 3 cultures compared.

§Serum of G. Caron Sr. (ref. 15).

it was established that input dengue virus decayed to below detectable levels at 48 h, the washing step was omitted⁵. Virus was assayed on LLC-MK2 monolayers by a plaque method⁶. Infection enhancement was defined as a significant increase in plaque counts observed in antibody-containing cultures compared with appropriate control groups (t test).

Comparative D2V growth observed in 20 paired experiments using rhesus PBL is shown in Fig. 1. In *M. mulatta* (rhesus monkey) or *M. irus* PBL cultures, virus titres were maximal after 72–120 h of culture but in human PBL peak virus growth occurred between 48 and 72 h. The enhancement potencies of selected dengue 1, 2, 3 or 4 antisera prepared in various vertebrate species are illustrated in Table 1. For each serum, neutralising antibody titres against homologous and dengue 2 viruses measured in LLC-MK2 cells are compared with D2V infection enhancing titres in PBL. Anti-dengue 1, 3 and 4 sera raised in man, monkeys, rabbits or mice produced a similar degree of D2V infection enhancement in monkey or human PBL over a broad dilution range. Monkey anti-dengue 2, with a homologous 50% plaque reduction neutralisation titre of 1:1280, neutralised D2V in PBL cultures at 1:100 but when diluted to and above the neutralisation end point, enhanced infection. Infection enhancement titres with homologous antisera were usually 20–100-fold greater than neutralisation titres; those in heterologous sera were usually 50–500-fold higher than the D2V neutralisation titre.

Over a period of 3 yr, PBL from 40 non-immune humans of all ages, from 25 *M. mulatta* and 22 *M. irus* have demonstrated D2V enhancement, but PBL obtained from two adult New Zealand rabbits, two guinea pigs and from two adult mice of each of eight strains (Swiss Webster, AKR, TO, DBA/TO, C₃H/10, Balb C, C₃H and DBA₂) failed to support replication in the presence of dengue 1–4 antibodies.

We have obtained evidence that the leukocyte supporting dengue replication *in vitro* is a mononuclear phagocyte (unpublished results). Thus, enhancement of dengue virus infection may be a special case of the opsonisation phenomenon. Non-neutralising antibody presumably provides a specific molecular 'ride' for an infectious dengue virion into the interior of a receptive phagocytic cell. It is well established that mononuclear phagocytes may serve as a genetically controlled determinant of viral pathogenicity; may be the initial site of viral replication, and may be a vehicle for systemic spread of and definitive host for viruses¹⁶. The failure of mouse, guinea pig and rabbit PBL to support dengue virus replication correlates with the insusceptibility of these species to dengue virus infection by the peripheral route¹⁷ and may provide yet another

example of pathogenicity determined by the ability of a virus to replicate in phagocytic cells. Mononuclear phagocytes are thought to be the only cells supporting replication in several viral infections, including Aleutian disease of mink¹⁸. Of particular interest to our report is the speculation that disease infection of monocytes results from the internalisation of virus-antibody complexes¹⁸.

If it is assumed that the right type and amount of dengue antibodies can increase the number of dengue-infected leukocytes *in vivo* and if enhanced cellular infection relates directly to disease severity, then the unusual age distribution of DSS in infants can be easily explained by the *in vitro* model described here. DSS is rarely seen in the first 3 months of life, then incidence rates rapidly increase, peak at 8 months and wane to low levels at 12 months (ref. 7). This suggests that in man as in PBL cultures, high concentrations of maternal dengue antibody may neutralise invading virus and protect the infant, but that after several months as antibody levels fall, infection enhancing activity predominates. In time this activity is lost.

The observation of antibody-enhanced virus infection in primate leukocytes is apparently new. Antibody enhancement of virus plaques, however, is well established in several systems. Hawkes and Lafferty noted enhanced plaque production when Murray Valley encephalitis virus (MVE) and rabbitpox viruses were inoculated on chick embryo fibroblast (CEF) monolayers in the presence of high dilutions of homotypic antisera¹⁹. Infection was mediated by avian IgG antibody and was completed within 30 min after virus-antibody mixtures were added to cell sheets. Mammalian antisera, although similar in potency, did not enhance. This unexplained result resembles a phenomenon described by Kjellén and Schlesinger²⁰. In their study, mixtures of vesicular stomatitis virus (VSV) plus diluted anti-VSV inoculated on CEF, resulted in titres 10–1,000-fold higher than when the same mixtures were applied to a continuous cell line. We found that CEF contain large numbers of phagocytic cells. Hence, enhancement of plaques on CEF and perhaps some instances of 'persistence' of virus infectivity in high dilutions of antisera may be earlier descriptions of the phenomenon reported here. In a related experiment, Azuma observed enhanced interferon production in mouse macrophages incubated with mixtures of Newcastle disease virus (NDV) and anti-NDV at dilutions above the neutralisation endpoint²¹. Virus replication was not measured, but interferon production was mediated by IgG, independent of complement, and required an active Fc terminal.

These reports suggest that enhanced viral infection of phagocytic cells by non-neutralising antibody may be a rather general biological phenomenon. This possibility

should be explored, particularly with viruses which exist in antigenically related groups.

We are now identifying immunoglobulin(s) mediating infection in PBL, studying the conditions which affect the reproducibility of *in vitro* antibody-mediated infection enhancement and the mechanism of infection of leukocytes by antibody-virus complexes. The availability of a simple system in which to elicit antibody-enhanced leukocyte infection may clarify the role of dengue-infected leukocytes in human pathogenic reactions.

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Colchicine inhibits phosphatidylinositol turnover induced in lymphocytes by concavalin A

RECENTLY colchicine has been shown to affect various membrane properties such as the lateral mobility of receptors and other surface membrane markers and the agglutination of different cell types. Many of the described phenomena also involve concanavalin A (con A); for example, phagocytosis by rabbit polymorphonuclear leukocytes (PMNs) leads to a selective depletion of con A binding sites which is prevented by colchicine¹, con A inhibition of lymphocyte immunoglobulin receptor capping is reversed by colchicine²⁻⁴ and con A-induced aggregation of PMNs, fibroblasts and hepatoma cells is inhibited by colchicine⁵⁻⁷. In addition to these effects on membrane properties, colchicine also has been shown to inhibit con A-induced lymphocyte transformation at an early stage in the sequence of events leading to thymidine incorporation and blast transformation^{8,9}. These various considerations led us to determine if colchicine could alter phosphatidylinositol (PI) turnover in lymphocytes since it is known that increased PI turnover is an early event induced by con A and other mitogenic lectins¹⁰⁻¹².

Table 1 shows that colchicine inhibits both unstimulated and con A-stimulated ³H-inositol incorporation into PI in a dose-dependent manner. At a concentration of 10⁻⁶ M the drug inhibited the con A increase in incorporation by 40%

to 66%. In other experiments (data not shown) 3 × 10⁻⁷ M colchicine caused slight but insignificant inhibition while at concentrations of 10⁻⁷ M or below it had no discernible effect. Colchicine also inhibited inositol incorporation into PI in unstimulated cells although to a lesser degree than when con A was also present. At a concentration of 10⁻⁶ M colchicine inhibited this basal incorporation in two out of four experiments (experiments 1 and 2, *P* < 0.01 and 0.1 respectively, Student's *t* test). At higher concentrations inhibition was both more pronounced and more consistent. Lumicolchicine was studied in one experiment only. At the concentrations used (10⁻⁶ M and 10⁻⁵ M) it had no effect on either basal or con A-stimulated inositol incorporation (data not shown).

While the results in Table 1 are very clear neither the mechanism by which colchicine inhibits inositol incorporation into PI nor the possible significance of this observation are immediately obvious. With respect to mechanism, it is well established that colchicine binds to tubulin and thereby prevents the formation of microtubules. It is possible, therefore, that an intact microtubule system is required for phosphatidylinositol turnover to occur. But it has been known for several years that colchicine also binds to particulate fractions in several cell types¹³⁻¹⁶ and that in the brain and thyroid gland, at least, the characteristics of this binding are indistinguishable from those of colchicine binding to tubulin. In addition, freeze-etch electron microscopy has shown that colchicine impairs the mobility of constituents of the alveolar membranes from *Tetrahymena pyriformis*¹⁷, presumably without altering microtubules. We favour the hypothesis that colchicine inhibits inositol incorporation through a direct interaction with tubulin or tubulin-like protein in membranes. It should be noted that this distinction between a colchicine action on microtubules or on membrane-bound protein may soon become irrelevant since there is increasing evidence of a very close association between microtubules and membranes. In rabbit ovarian granulosa cells, for example, microtubules are seen perpendicular to and directly beneath con A caps while fluorescein-labelled colchicine is localised to the cap region¹⁸. Similarly, Berlin and his colleagues have recently applied the technique of resonance energy transfer between fluorescent chromophores to demonstrate a close association between tubulin and membrane components in rabbit polymorphonuclear leukocytes^{19,20}.

Another possible explanation for the present results, namely that colchicine directly affects an enzyme or enzymes involved in PI turnover, seems unlikely since colchicine has been shown not to affect the enzymatic activity of a soluble PI phosphodiesterase purified from hog thyroid gland²¹. It is of considerable interest, nonetheless, that this phospholipase C-type enzyme, which forms cyclic inositol phosphate as one of its products, has been shown to be associated with tubulin^{21,22}. Finally, it should be mentioned that the trivial explanation, inhibition by colchicine of con A binding, has been eliminated by other investigators⁸.

Before considering the relevance of the present observations it is necessary to consider the significance of increased inositol incorporation into PI. It has been known for many years that numerous stimuli cause the increased incorporation of both phosphate and inositol into phosphatidylinositol (for review, see refs 23-25). In almost all tissues this incorporation reflects the turnover of the phosphorylinositol portion of the PI molecule rather than *de novo* synthesis. The enzyme initially activated by the stimuli which lead to PI turnover is of the phospholipase C type and is capable of forming D-myo-inositol 1,2-cyclic phosphate as one of its water-soluble products^{26,27}. Four years ago Michell and Lapetina suggested that this compound acts as an intracellular second messenger²⁷. Increased PI turnover occurs almost immediately after the addition of con A or other

Table 1 Effects of concanavalin A and colchicine on ^3H -inositol incorporation into PI

Additions		Expt 1	Incorporation (counts per 10 min $\pm$ s.d.)			Expt 4
—		1,005 $\pm$ 44	679 $\pm$ 34	1,212 $\pm$ 56	1,962 $\pm$ 198	
Colchicine	10^{-4} M	823 $\pm$ 130	358 $\pm$ 41	751 $\pm$ 54	1,191 $\pm$ 42	
Colchicine	10^{-5} M	910 $\pm$ 49	485 $\pm$ 56	1,159 $\pm$ 58	1,450 $\pm$ 193	
Colchicine	10^{-6} M	854 $\pm$ 21	540 $\pm$ 83	1,258 $\pm$ 73	2,039 $\pm$ 153	
Con A	20 $\mu\text{g ml}^{-1}$	1,707 $\pm$ 77	1,129 $\pm$ 12	2,860 $\pm$ 196	3,581 $\pm$ 166	
Con A + colchicine	10^{-4} M	1,089 $\pm$ 59	555 $\pm$ 56	1,131 $\pm$ 37	1,860 $\pm$ 48	
Con A + colchicine	10^{-5} M	1,200 $\pm$ 180	767 $\pm$ 71	1,599 $\pm$ 17	2,250 $\pm$ 26	
Con A + colchicine	10^{-6} M	1,273 $\pm$ 160	829 $\pm$ 74	1,893 $\pm$ 113	2,740 $\pm$ 212	

Four to five $\times 10^6$ human peripheral lymphocytes isolated by density gradient centrifugation were incubated in triplicate in 1.0 ml Eagle's minimum essential medium containing 10% foetal calf serum with or without colchicine for 30 min at 37 °C. Con A and ^3H -inositol (approximately 1 μCi) were added and the incubation continued for 100 min after which the cells were pelleted and washed twice with medium. After addition of 7.5 ml chloroform-methanol (1:2) the cells were sonicated. 2.5 ml each of H_2O and chloroform were then added and the samples left overnight. Following centrifugation the chloroform phase was dried in scintillation vials, the residue dissolved in scintillation solution and the ^3H content determined in a liquid scintillation counter. The ^3H was shown to be localised to the PI fraction by ascending chromatography using silica gel loaded paper and both basic and acidic solvents. In preliminary experiments con A-enhanced ^3H -inositol incorporation into PI at concentrations of 2.5–20 $\mu\text{g ml}^{-1}$. 20 $\mu\text{g ml}^{-1}$ con A was used in subsequent experiments since it produced the greatest enhancement without causing any cytotoxicity as determined by Erythrocin B exclusion.

mitogenic, but not non-mitogenic, lectins to lymphocytes and continues for as long as the lectin is present^{10,11}. It has been proposed that this enhanced PI turnover is an essential step in the mechanism of lymphocyte activation. We consider that the present demonstration of colchicine inhibition of PI turnover both explains the recent observation^{8,9} that colchicine inhibits concanavalin A-induced lymphocyte transformation at an early stage and also provides additional strong support for the hypothesis that increased PI turnover is required for lymphocyte transformation to occur.

The significance of the present observation with respect to colchicine effects on lateral mobility within membranes and cell agglutination is difficult to assess at present. Both phagocytosis, which initiates movement within membranes^{1,28}, and anti-immunoglobulin antisera are stimuli which increase PI turnover in PMNs^{29–31} and lymphocytes¹² respectively. It is certainly possible that membrane changes influencing mobility occur simultaneously with or as a later result of this increased PI turnover and that colchicine alters movement by its effect on this parameter.

In summary, colchicine has been found to inhibit both basal and concanavalin A-stimulated incorporation of ^3H -inositol into phosphatidylinositol. We consider this effect explains the early inhibition by colchicine of con A-induced thymidine incorporation into lymphocytes and has possible relevance for colchicine effects on membrane mobility and agglutination. It will be important to determine if this newly described action of colchicine occurs in cell types other than lymphocytes and if it is shared by other microtubule-active agents.

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Asymmetrical distribution of microvilli in cytochalasin B-induced pseudocleavage of mouse oocytes

CYTOCHALASIN B, a fungal alkaloid first described by Carter¹, has diverse effects on cultured cells, including inhibition of morphogenetic and movement processes, and interference with hormone mediated cellular response². It also induces various cultured mammalian cells to segregate the nucleus into an evagination of the plasma membrane which occasionally breaks away from the cell body³. Although some of the biological effects of cytochalasin B have been attributed to the disruption of microfilamentous structures found in cells, the mechanism of action of the drug remains unclear⁴. We have found that mouse oocytes cultured in the presence of cytochalasin B divide into two compartments; a process we called 'pseudocleavage'⁵. We now report, using scanning electron microscopy, that although one of the oocyte compartments is covered with microvilli, the other is smooth. This suggests that cytochalasin B triggers a redistribution of cell surface elements in the mouse oocyte.

Approximately 15% of mouse oocytes cultured for 16 h in medium containing cytochalasin B (CCB, 5 $\mu\text{g ml}^{-1}$) underwent pseudocleavage (Fig. 1a and d). This percentage was increased several-fold by the addition of db cyclic AMP (0.2 mM) to the medium⁶ or by using oocytes which failed to resume meiosis

when cultured for 3 h in medium without CCB (Fig. 2). The latter experiments show that exogenous db cyclic AMP is not required for CCB-induced pseudocleavage.

To compare the topography of normal and pseudocleaved oocytes, isolated mouse oocytes were cultured in the presence of either db cyclic AMP (0.2 mM) alone or db cyclic AMP (0.2 mM) and CCB ($5 \mu\text{g ml}^{-1}$) for 16 h, then fixed and processed for scanning electron microscopy. The surface of oocytes cultured in the presence of db cyclic AMP alone was uniformly covered with microvilli (Fig. 1*b* and *c*), resembling that of oocytes cultured without db cyclic AMP. On the other hand, the surface of one of the two compartments resulting from pseudocleavage was covered with microvilli, while the other was comparatively smooth (Fig. 1*e* and *f*).

Because microvilli are uniformly distributed on the surface of mouse oocytes cultured in the presence or absence of db cyclic AMP, pseudocleavage apparently involves extensive topographical reorganisation of the oocyte. Although the mechanism of action of CCB is not clear⁴, we suggest that the surface asymmetry observed here is due to interaction of CCB with the oocyte's cortical contractile apparatus¹⁰. In this connection, several investigators have suggested that peripheral membrane

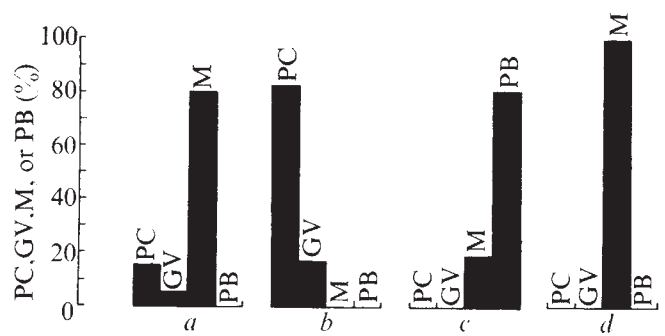


Fig. 2 Analysis of oocyte behaviour in different culture conditions. In all cases oocytes were cultured for 16 h then scored microscopically as pseudocleaved (PC), germinal vesicle (GV), metaphase I (M), or polar body (PB) oocytes. *a*, oocytes cultured continuously in the presence of CCB ($5 \mu\text{g ml}^{-1}$). *b-d*, oocytes were cultured in medium for 3 h, then those retaining an intact GV were transferred to medium containing CCB ($5 \mu\text{g ml}^{-1}$) (*b*), and oocytes which had undergone GV breakdown were transferred either to medium (*c*) or to medium containing CCB ($5 \mu\text{g ml}^{-1}$) (*d*). Each sample contained 50 or more oocytes.

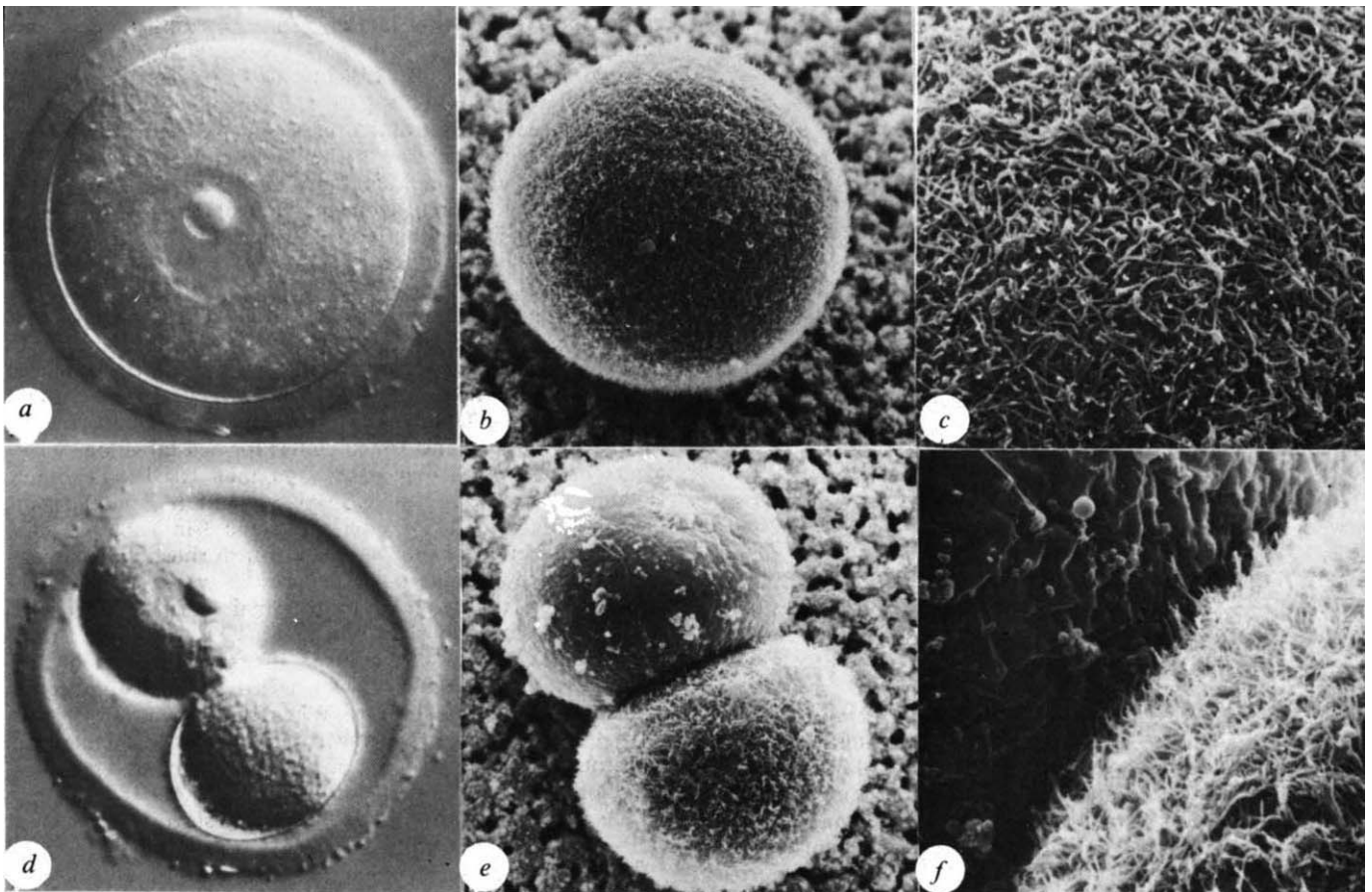


Fig. 1 Photomicrographs of mouse oocytes after *in vitro* culture. Oocytes were obtained by puncturing ovaries dissected from adult (8–12 weeks old) virgin female Swiss mice (CD-1, Charles River)⁶. Oocytes containing an intact germinal vesicle (GV) and free of cumulus cells were collected and washed in a chemically defined medium⁷ and cultured in this medium under paraffin oil at 37°C in a humidified atmosphere of 5% CO_2 in air. Approximately 85% of the oocytes underwent GV breakdown within 3 h and, of these, approximately 80% subsequently emitted polar bodies. In the presence of db cyclic AMP (dbcAMP, 0.2 mM) <5% of the oocytes resumed meiosis, as shown by the retention of an intact GV and diffuse chromatin even after 16 h (refs 8 and 9). Air-dried chromosome spreads¹⁷ of cultured oocytes confirmed that the oocytes were arrested in the dictyate stage of meiosis. *a, d*, Light microscopy of live oocytes cultured for 5 h with either dbcAMP (0.2 mM) (*a*) or dbcAMP (0.2 mM) and CCB ($5 \mu\text{g ml}^{-1}$) (*d*). Light microscopy was performed using a Zeiss Photomicroscope II equipped with Nomarski differential-interference optics (*a*, $\times 690$; *d*, $\times 650$). *b, c, e, f*, scanning electron microscopy of fixed oocytes cultured for 16 h with either dbcAMP (0.2 mM) (*b, c*) or dbcAMP (0.2 mM) and CCB ($5 \mu\text{g ml}^{-1}$) (*e, f*). Oocytes were fixed with 2% buffered glutaraldehyde and zona pellucidae were removed by brief treatment with pronase (0.5%). Oocytes were collected on polylysine-coated silver membrane filters (0.8 μm porosity, Flotronics) by gentle suction, post-fixed with 1% buffered OsO_4 for 30 min at 4°C , washed with buffer, dehydrated through a graded series of acetones, and critical point dried out of liquid CO_2 . Filters were mounted on aluminium studs, coated with gold and palladium in a sputtering device (Techniques Inc.), and examined in an ETEC Autoscan scanning electron microscope (*b*, $\times 690$; *c*, $\times 5600$; *e*, $\times 900$; *f*, $\times 5400$).

components, such as actin and tubulin, influence the surface properties of cells¹¹⁻¹³. Sundqvist and Ehrnst¹⁴ have reported that exposure of several types of cultured cells to CCB resulted in an asymmetrical redistribution of microfilaments and cell surface elements. Pseudocleavage may represent an analogous response to CCB by the oocyte.

It is interesting, in view of our results, that other investigators have observed a pronounced asymmetrical distribution of microvilli over individual blastomeres during early mammalian embryogenesis^{15,16}. The changes in surface organisation seem to represent a very early morphogenetic event in the embryo. CCB-induced pseudocleavage may represent an aberrant induction of events that would normally occur later in development.

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Malignant transformation of rat embryo fibroblasts by herpes simplex virus types 1 and 2 at suboptimal temperature

HERPES SIMPLEX VIRUS (HSV) transforms cultured mammalian cells in three systems, involving (1) ultraviolet-irradiated virus¹⁻⁴, (2) increased incubation temperature^{5,6} and (3) temperature-sensitive mutants^{7,8}. Malignant transformation by HSV has been demonstrated for hamster, rat and mouse cells, using three systems^{4,8-12}. We now report a further system, involving the use of a suboptimal temperature. This method is relatively convenient and seems to be applicable to various rat cell lines.

Sprague-Dawley rat single embryo cells (REF-1-76) were derived from a single clone, and grown as described before^{12,13}. The cells were fibroblasts as shown in Fig. 1a. The normal REF-1-76 cells were established and propagated in tissue culture and so far they have not revealed any variability in the tests used in our laboratory in various tissue culture passages, even individual ones.

Three HSV strains, HSV type 1-Thea, HSV type 2-Müller and HSV type 2-HG-52, were propagated on human fibroblast cells and titred by a plaque assay^{21,14}. The REF monolayer cultures in the 5th tissue culture passage were infected with HSV-1 or 2 at a multiplicity of infection (MOI) of 5

plaque-forming units (PFU) per cell. After 1 h of incubation at 37 °C the infected cells were shifted down to a suboptimal temperature of 20 °C. The cells were incubated for 1 h at 37 °C, washed five times with Hanks' solution, refed with conditioned medium¹² and divided into two parts. One part was incubated at 37 °C and the second part was shifted down to a suboptimal incubation temperature of 20 °C. Parallel cultures were mock-infected and incubated in the same way as the infected cultures and served as a control. Infected cells developed a typically HSV-induced cytopathic effect (CPE) and were destroyed when incubated at 37 °C.

Ninety per cent of the cells survived this incubation at 20 °C. The floating cells were collected by low-speed centrifugation (800g at 4 °C for 10 min), and transferred by trypsinisation to cell culture bottles. The appearance of floating cells seems to have been due to proliferating cells, which after treatment with 0.25% trypsin at 25 °C and seeding for the next passage in tissue culture lost their floating property and grew in monolayers. The monolayers and floating cells were then shifted separately up to 37 °C and maintained at this temperature. Two weeks later new cell colony formation was observed in the case of the floating cells which had been seeded and subsequently incubated at 37 °C. Transformation efficiency was approximately one colony per 10⁵ original floating cells, corresponding to the formation of one colony per 1 × 10⁶ HSV-infected rat embryo fibroblast cells. In contrast, the monolayer cells developed a typically HSV CPE and completely degenerated.

Mock-infected control cultures survived our procedure without forming foci and without producing floating cells. The new cell colonies were morphologically different from each other and different from the control cultures (Table 1). The new cells were either epithelia-like (Fig. 1b) or spindle-like (Fig. 1c), as reported for HSV-transformed REF cells^{7,12}. Some clones were isolated by being picked from foci of colonies which had originated from floating cells and become established in tissue culture. As Table 1 shows, HSV-1-Thea was used for transformation of the isolated cell clone REF-T-20; HSV-2-Müller was used for REF-T-20-2, and HSV-2-HG-52 was used for REF-T-20-3. Two of the isolated HSV-transformed clones formed infectious virus after three to five subpassages and then lysed. The other isolated clones were stable in tissue culture and did not produce infectious HSV (clones 1, 2 and 3 in Table 1). The plating efficiency of normal and transformed cell clones, determined by standard procedures¹⁵, was two or three times greater than that of the original, normal REF cells (Table 1). Using published procedures¹⁶, we also found that the modal chromosome number of the transformed cell clones was greater than 42, which is the modal number of primary rat cells (Table 1). When the extent of colony formation in soft agar was measured¹⁷, both the spindle-like transformed cell clones grew in soft agar, but the epithelia-like clone did not (Table 1).

The capacity of the isolated HSV-transformed clones to

Fig. 1 Photomicrographs of normal and transformed rat embryo fibroblast cultures: a, normal rat embryo fibroblast (REF) culture; b, transformed rat embryo fibroblast culture, epithelia-like clone and c, spindle-like clone; phase contrast.

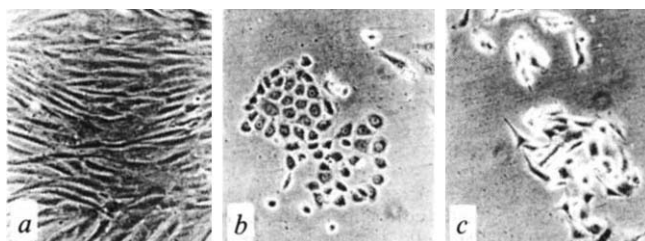


Table 1 Properties of isolated HSV-transformed rat embryo fibroblast cells at suboptimal temperature (20 °C)

Cell types and number of clones	Virus	Morphology of cells	Plating	Modal no. of chromosomes	Growth in soft agar	Tumour induction	
			Efficiency of cells %			No. of cells inoculated	Efficiency
REF normal	Mock	Fibroblast-like	20	42	—	1×10^8	0/10*
REF-T-20-1	HSV-1-Thea	Spindle-like	58	89	+	1×10^6	10/10
REF-T-20-2	HSV-2-Müller	Spindle-like	52	97	+	1×10^6	6/6
REF-T-20-3	HSV-2-HG-52	Epithelia-like	40	66	—	1×10^7	0/10

*No. of tumours per no. of animals inoculated.

induce tumours in rats was evaluated. Newborn Sprague-Dawley rats (a closed but not strictly inbred colony), 1–2 d old, were inoculated with either normal or transformed REF cells, using 1×10^6 to 1×10^8 cells per rat. Animals were observed at least twice weekly for the development of palpable tumours at the site of inoculation. Table 1 shows that the two spindle-like transformed cell lines were highly tumorigenic in syngeneic animals. The induced tumours grew very rapidly and induced metastases in the lung. Histopathologically, the tumours were immature spindle-cell sarcomas. No regression of tumour growth was observed. The tumours could be transplanted successfully directly from animal to animal, or could be explanted and grown in tissue culture. Such cell cultures had the same morphological characteristics as the cells originally inoculated.

The detection of HSV antigens in the isolated cell clones and cell lines derived from the tumours was investigated by the indirect method of immunofluorescence, using hyperimmune rabbit antisera. The *in vitro* transformed cell clones with spindle-like morphology showed cytoplasmic HSV immunofluorescence. In contrast, the epithelia-like cell clone showed very weak fluorescence (Table 2). Cells derived from

cells²⁰ showed that in all cases, including cell lines derived from rat tumour biopsies, C-type virus particles were not detectable. Transformation at suboptimal temperature was repeated with two independently isolated rat embryo fibroblast cell cultures in three independent experiments and gave similar results in all cases.

In conclusion, we have shown that rat embryo fibroblasts inoculated with HSV-1 or 2 were transformed when the cells had been incubated at the suboptimal temperature of 20 °C for the first 3 weeks after infection. The transformed cell clones with spindle-like morphology were highly tumorigenic. In all control experiments the mock-infected cultures showed 20% plating efficiency and the modal chromosome number was 42. The cultures did not grow in soft agar and did not induce tumours in syngeneic animals. The mechanism of transformation at suboptimal temperature remains unclear and it is open to question whether or not the presence of part of the HSV genome is required for the maintenance of the transformed state.

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Table 2 Detection of HSV-antigens in HSV-transformed and normal rat embryo fibroblast cells and sensitivity to superinfection with HSV types 1 and 2

Immunofluorescence			HSV-Inoculum					
Cell types	Cytoplasmic	Nucleic	HSV-1 (Thea) [PFU per dish]*			HSV-2 (Müller) [PFU per dish]		
			1×10^2	1×10^3	1×10^4	1×10^2	1×10^3	1×10^4
			No. of plaques per dish			No. of plaques per dish		
REF control	—	—	109	TNC	total CPE	103.5	TNC	total CPE
REF-T-20-1	+	—	—	—	—	—	—	—
REF-T-20-2	+	—	—	—	—	—	—	—
REF-T-20-3	(+)	—	24	196	TNC	1	15	153

TNC, too numerous to count.

*Petri dishes contained 5×10^5 cells.

the tumours also exhibited HSV cytoplasmic immunofluorescence comparable with that of the original *in vitro* transformed cells. The isolated transformed cell clones were checked for resistance against HSV-superinfection. Monolayers of 5×10^5 cells from each clone were infected with 1×10^2 , 1×10^3 and 1×10^4 PFU of HSV-1-Thea or HSV-2-Müller and overlaid with conditioned medium containing 0.5% carboxymethyl-cellulose as described previously^{12,14}. After 3 d of incubation at 37 °C in 5% CO₂ the cultures were fixed with 5% formaldehyde in phosphate-buffered saline and stained with 1% crystal violet and virus plaques were counted (Table 2). The spindle-like transformed cells, in contrast to normal and epithelia-like transformed cells, were completely resistant to superinfection with HSV types 1 and 2 in these conditions. The superinfectability of the epithelial cell clone was considerably reduced, compared with REF cells, usually by a factor 10 against HSV-1 and 10^2 against HSV-2.

RNA-directed DNA polymerase^{18,19} was assayed to investigate the possible presence of oncornavirus in the transformed cell clones, the tumour material and cell lines derived from the tumours (manuscript in preparation). The standard mixed culture cytopathogenicity test with XC

HSV-1 Thea and HSV-2 Müller; Dr F. Deinhardt for interest and for HSV-2-HG-52, and Drs A. Fried and C. H. Schröder for helpful discussions.

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Secretion of hCG- α subunit and hCG by HeLa strains

IN recent letters^{1,2}, we have independently reported seemingly different secretion patterns of human chorionic gonadotropin (hCG) and its alpha (hCG- α) and beta (hCG- β) subunits by certain HeLa strains. These letters were further discussed in an article in *News and Views*³. Ghosh and Cox¹ found that HeLa 65 and HeLa 71 secreted a product with activity in an 'hCG- β ' immunoassay (detecting hCG and/or hCG- β), and that this secretion was greatly stimulated by butyrate; hCG- α was not measured. Lieblisch *et al.*² reported that HeLa CCL 2, 2.1 and 2.2 secreted very large quantities of hCG- α but no detectable amounts of hCG or hCG- β (<0.2 ng ml⁻¹). Two questions were raised: what was the nature of the immunoactive material in the hCG- β assay, and could the differences between the two laboratories be attributed to the known variability among HeLa strains? We have collaborated in an attempt to answer these questions, and we confirmed strain differences in hCG- α secretion in HeLa cells.

HeLa 65 and HeLa 71 were grown in increasing concentrations of sodium butyrate at New York University, and the media were analysed for hCG, hCG- β and hCG- α at the National Institutes of Health. In separate, specific radioimmunoassays (RIA), we have identified the 'hCG- β ' immunoactivity as complete hCG; no free hCG- β was found (<1 ng ml⁻¹). Both HeLa strains secreted much larger quantities of free hCG- α than complete hCG (Table 1), but not sufficient hCG- α to

Table 1 Basal and butyrate-stimulated secretion* of hCG- α subunit and hCG in two HeLa strains

	Butyrate concentration (mM)	Cell no. ($\times 10^6$)	hCG (pmol per 10^6 cells per day)	hCG- α (pmol per 10^6 cells per day)
HeLa 65	0	17	0.01	<0.03
	1	11	0.6	2.0
	5	2.2	1.1	16
HeLa 71	0	22	0.02	15
	1	8.2	0.4	430
	5	0.3	3.0	1,450

*HeLa cells grown for 3 days in Waymouth's medium containing 10% foetal calf serum with additions of sodium butyrate as shown.

produce significant cross reaction in the hCG RIA. Sodium butyrate stimulated both HeLa strains to increase markedly the secretion of both proteins, with the secretion of hCG- α far in excess of hCG. Although the relative stimulation by butyrate of hCG and hCG- α were within an order of magnitude (~ 100 -500-fold), the absolute levels of hCG- α secretion exceeded complete hCG by 15-fold in HeLa 65 and 500-fold in HeLa 71. These differences between HeLa 65 and HeLa 71 in basal and butyrate-stimulated secretion of hCG and hCG- α , and the previously reported² differences among HeLa 2, 2.1 and 2.2 in basal hCG- α secretion may be functional counterparts of the well-described karyotypic differences among strains of HeLa.

We have tested butyrate stimulation of another cell line producing α subunit ectopically. Preliminary studies in ChaGo (ref. 4), derived from a primary carcinoma of the lung, also show significant butyrate stimulation of hCG- α secretion rates.

Demonstration of butyrate stimulation in a second ectopic protein-producing cell line underlines the potential of this reagent in the study of biosynthetic mechanisms.

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Effects of immunisation against luteinising hormone releasing hormone on reproduction of the marmoset monkey *Callithrix jacchus*

LUTEINISING hormone releasing hormone (LHRH) is a decapeptide secreted by the hypothalamus and transported through the hypophyseal portal system to the anterior pituitary gland where it acts to stimulate gonadotrophin release¹. Selective neutralisation of this releasing factor by specific antibodies has been achieved in rodents²⁻⁴, but there has been no report of similar work on primates. Immunisation against hypothalamic releasing hormones is a potentially suitable method for selective control of the secretion of anterior pituitary hormones. In particular, interference with LHRH release might make possible a new approach to contraception. We describe here how antibodies to LHRH were raised in the marmoset, *Callithrix jacchus*, and outline the subsequent effects on gonadal activity and pituitary function.

Ten adult marmosets, five male and five female, were immunised with synthetic LHRH conjugated to bovine serum albumin (BSA) using the carbodiimide technique⁵. Each animal received eight intradermal injections of the conjugate emulsified in Freund's adjuvant (FCA), followed by *Bordetella pertussis* vaccine as an additional adjuvant. Four further animals, two male and two female, were injected with BSA alone in FCA, together with *B. pertussis* vaccine, and served as controls. Each animal received a booster injection approximately 10 weeks after primary immunisation.

Heparinised blood samples were collected once a week from the femoral vein, centrifuged immediately and plasma was stored at -20°C for subsequent determination of hormone and antibody levels. Testicular and uterine dimensions were also measured once weekly, using calipers. Progesterone, LH, and testosterone were measured by radioimmunoassay techniques described elsewhere⁶⁻⁸. The presence of antibody to LHRH was assessed by measuring the percentage binding of approximately 10 pg radioiodinated LHRH by plasma at a dilution of 1:500.

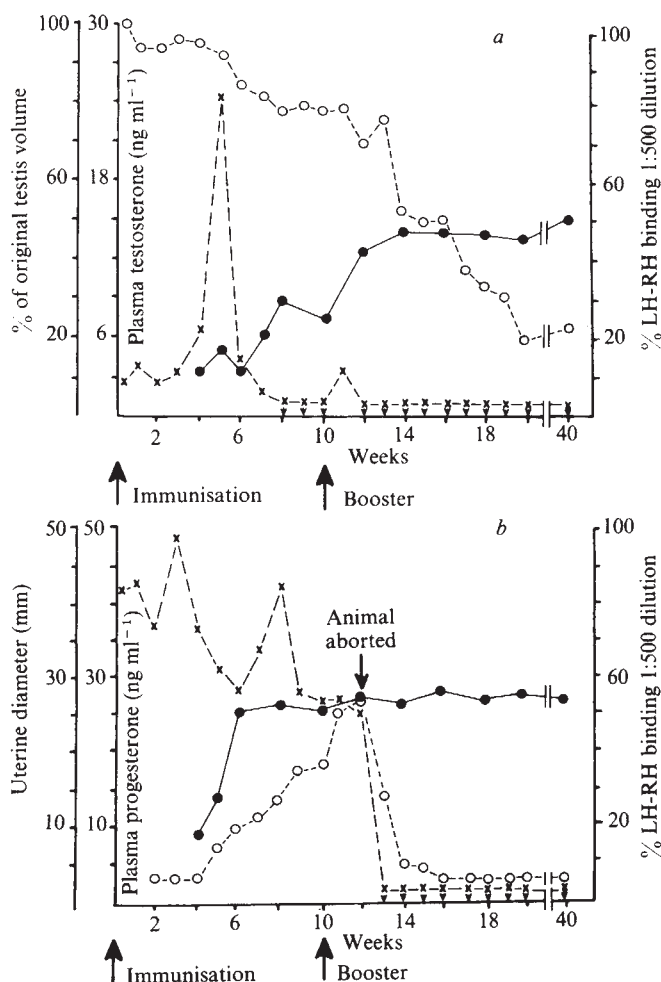


Fig. 1 Effects of immunisation against LHRH in the marmoset. *a*, Testosterone concentration (x), testis volume (O) and antibody titre (●) in a male; *b*, progesterone concentration (x), uterine diameter (O) and antibody titre (●) in a female. Arrows indicate undetectable.

No antibodies to LHRH were produced by the control animals and their gonadal activity was normal. Although all LH-RH-immunised animals produced antibodies, in four cases the titres were low (< 15% binding).

Of the six animals which developed high antibody titres (47–57% binding) all three males showed marked testicular atrophy and suppression of testosterone secretion, while all females developed inactive ovaries and became acyclic.

Figure 1 illustrates the effects of immunisation against

LHRH in one male and one female marmoset. In the male (Fig. 1*a*) antibody titres increased gradually during the first 10 weeks, but plasma testosterone levels were not depressed until after the booster injection, when the concentration of antibodies increased markedly. Plasma testosterone then decreased to undetectable levels (< 1 ng ml⁻¹) and the testes became softer and smaller, eventually shrinking to less than 25% of their original volume.

The female (Fig. 1*b*) was about 2 weeks pregnant at the time of immunisation and showed a more rapid build up of antibody titres during the following 6 weeks, while pregnancy continued normally. The antibody titre had reached a maximum by week 8 and the booster produced no further increase. Progesterone then began to decrease, dropping rapidly at week 12 to undetectable levels as the animal aborted. Progesterone concentrations were minimal (< 5 ng ml⁻¹) for up to 40 weeks after immunisation, showing that ovulation had ceased.

Basal LH levels in normal marmosets fluctuate around the limit of sensitivity of the LH assay (20 ng ml⁻¹ LHRP-1 equivalent). Consequently, it was not possible to detect any decrease in LH concentrations after immunisation, and these results have been omitted from this report.

Histological sections of the control testes showed normal spermatogenesis, but in the three males with high levels of antibody (52–55% binding) there was marked involution of the seminiferous tubules, and spermatogenesis was severely impaired (Table 1). Interstitial cells were greatly reduced in size and contained condensed nuclei. Although seminiferous tubules in animals with low antibody titres (< 15% binding) were also reduced in size, they were considerably larger than the tubules in animals with high antibody titres, and spermatogenesis had not been affected. Ovaries in female marmosets with high antibody titres (47–57% binding) were much reduced in size, and although follicular development could be seen there was no luteal tissue. Ovaries in females with low antibody titres (< 15% binding) were not significantly reduced in size, and contained both follicular and luteal tissue (Table 1).

The effect of immunisation on the response of the pituitary to exogenous LHRH was also studied. Plasma LH levels were measured immediately before, and 45 min after intramuscular injections of various doses of LHRH (4 µg, 10 µg and 50 µg) to animals 40 weeks after immunisation (Table 2). Animals with high antibody titres showed a much reduced response at all doses compared with animals with low antibody titres, and with controls. A dose response relationship was demonstrated by high titre animals, whereas animals with low antibody titres showed a maximal response to all three doses of LHRH. Even in animals with high antibody titres that had been immunised for 40 weeks and were infertile, the pituitary gonadotrophs were still able to respond to the releasing hormone. An LHRH test on immunised animals may provide a better

Table 1 Ovarian and seminiferous tubule measurements in control and immunised marmosets

Group	Tubule diameter (µm) Mean ± s.d.	Ovarian size length (mm) × breadth (mm) Mean ± s.d.
Control	279.5 ± 21.7	61.4 ± 2.2
LHRH-immunised high titres	132.2 ± 9.9	12.4 ± 1.7
LHRH-immunised low titres	245.1 ± 21.1	47.6 ± 3.9

In each group 20 tubule diameters from two males were measured. The mean tubular diameter in each group of immunised animals was significantly smaller than that in control animals ($P < 0.001$; Student's *t* test). Laparotomy was performed on two females in each group and the right ovaries were measured across their longest and shortest axes. The product of these two measurements was taken as a rough indication of ovarian size. Ovaries in animals with high antibody titres were significantly smaller than those in the controls ($P < 0.001$; Student's *t* test). There was no significant difference in ovarian size between control animals and animals with low antibody titres.

Table 2 Response to LHRH in control and immunised marmosets

	4µg	Male 10µg	50µg	4µg	Female 10µg	50µg
LHRH-immunised high titre (6)	0†	14	23*	7*	22	28*
LHRH-immunised low titre (4)	79	63*	67	72	68*	63*
Control (2)	83	—	—	87*	—	—

Plasma LH concentrations (ng ml⁻¹ LHRP-1 equivalent) were measured before and 45 min after administration of LHRH, and the net increase (Δ LH) is shown. The total number of animals used is indicated in parentheses.

* Pre-injection levels were undetectable (< 20 ng ml⁻¹) and to calculate Δ LH, a value of 20 ng ml⁻¹ was substituted. A slight underestimation of Δ LH values has therefore resulted.

† Both pre- and post-injection levels were undetectable.

indication of the effectiveness of immunisation than measurement of antibody titres.

These results demonstrate that immunisation against LHRH is an effective way of suppressing gonadal activity in the marmoset. Although the necessity for Freund's adjuvant and the rather drastic effects of complete inhibition of LHRH preclude its acceptability as a method of human fertility control at the moment, it may still be possible to develop a new approach to contraception by LHRH inhibition, achieved either immunologically, or by the use of competitive LHRH antagonists. Total suppression of the testis would be necessary in the male to ensure infertility, and this would probably be undesirable. In the female, however, it may be possible to suppress the ovary to a level which would effectively prevent ovulation, but would still allow some follicular development and oestrogen secretion.

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The carcinogen ethionine elevates progesterone levels

ETHIONINE is a potent carcinogen, but little is known about its mode of action. It is known that it does not react extensively with DNA¹. It may be an ubiquitous carcinogen, as it is synthesised by *Escherichia coli*² and several other microorganisms, so studies of its mode of action are important. We reported recently a novel effect of ethionine in the immature chick³. The oviducts of chicks previously stimulated with oestrogen behaved after the administration of ethionine as if they had received a second stimulation with oestrogen or progesterin^{4–6}. The egg white proteins, ovalbumin and conalbumin were synthesised and distinct morphological changes, characteristic of stimulation by oestrogen or progesterin appeared. It was thus not clear whether ethionine (or its metabolic product) was affecting steroid hormone receptors⁷ directly, or interfering in some way with steroid hormone metabolism to produce elevated levels of endogenous oestrogen or progesterin. We report here that ethionine or its sulfoxide are unique in inducing in the chick oviduct the effects cited above; analogues were inactive. We also report that the administration of ethionine elevates serum progesterone levels over 10-fold, to a concentration which may be sufficient to account for the profound changes in the immature oviduct.

We used the procedure developed in the laboratories of O'Malley^{8,9} and Schimke^{4,5,10}. Oestrogen administration to the immature chicks results in extensive cytodifferentiation of the primitive oviduct and synthesis of cell-specific proteins. Ovalbumin synthesis is first detected approximately 18 h after oestrogen stimulation, and after 7–10 d of daily stimulation it accounts for 50–60% of total protein synthesis (primary stimulation)¹¹. Discontinuation of oestrogen administration is accompanied by a gradual loss in cell-specific protein synthesis. Ovalbumin synthesis is not detectable after 15–20 d of oestrogen

Table 1 Effect of analogues and metabolites of ethionine on induction of ovalbumin synthesis in immature chick oviduct

Treatment	¹⁴ C-amino acids incorporated, per mg soluble protein (c.p.m. × 10 ⁻⁵)	¹⁴ C-amino acids incorporated into ovalbumin, per mg soluble protein (c.p.m. × 10 ⁻⁵)	Ovalbumin synthesis, % of total protein synthesis
None	1.84	ND	0
L-Ethionine	2.43	0.59	24.3
L-Ethionine sulfoxide	1.91	0.327	17
	1.57	0.062	4
L-Ethionine sulphone	1.03	ND	0
	1.19	ND	0
N-Acetyl-L-ethionine	1.42	ND	0
	1.47	ND	0
S-Propylhomocysteine	1.03	ND	0
	1.46	ND	0

Four day old chicks were injected with 1 mg of oestradiol 17- β -benzoate in sesame oil for 10 d. After a withdrawal period of 4 weeks, the chicks were injected (in groups of eight), intraperitoneally (i.p.) with a 1-ml suspension, in water, of L-ethionine or its analogue and metabolites (0.25 mg per g body weight) and adenine (0.12 mg per g body weight) for 5 d. On the sixth day the animals were sacrificed, the oviducts removed, the magnum portions of the oviduct freed of adjoining tissue and cut into small pieces. The magnum explants were incubated *in vitro* in antibiotic free medium containing labelled amino acids¹². Tissue was incubated in 2 ml of medium in 25-ml Erlenmeyer flasks for 3 h. Each flask contained tissue from three oviduct magna. Ovalbumin synthesis in the magnum explant was determined by specific immunoprecipitation as described before^{13–16}. L-ethionine was from Sigma, L-ethionine sulfoxide, L-ethionine sulphone and N-acetyl-L-ethionine were from Cyclo. S-propylhomocysteine was synthesised in our laboratory¹⁷. ND, not detected.

Table 2 Elevation of progesterone levels in immature chicks by ethionine

Treatment	Ovalbumin synthesis in oviducts, % of total protein synthesis	Serum progesterone (ng per 100 ml)	Serum 17- β oestradiol (ng per 100 ml)	Total serum cholesterol (mg per 100 ml)
None	ND	29	10	103
Ethionine + Adenine				
3 d	ND	40	8	155
4 d	6	153	7	150
5 d	16	285	3	114
Oestradiol 17- β benzoate, 2 d	35	40	448	361
Progesterone, 2 d	34	523	7	113

After 10 d of primary oestrogen stimulation chicks were withdrawn for 21 d and (in groups of eight) injected i.p. with ethionine and adenine as described for Table 1. Oestradiol or progesterone (2 mg per chick, in sesame oil) were injected intramuscularly. At the times specified, blood was collected by cardiac puncture and the separated serum was stored frozen until analysed. Analyses for progesterone¹⁸, 17- β -oestradiol¹⁹ and total cholesterol²⁰ in serum were performed by Laboratory Procedures, Upjohn. The values are the average of duplicate determinations. These experiments were repeated with another group of chicks with essentially the same results (data not shown). Ovalbumin synthesis was determined in duplicate and the values are an average. There were some variations in the time course and degree of induction of ovalbumin synthesis and progesterone by ethionine.

withdrawal (withdrawn chicks). Administration of oestrogen or progesterone^{4,5} or ethionine³ to withdrawn chicks results in rapid restoration of cell-specific protein synthesis in the chick oviduct.

To ascertain whether ethionine analogues or its metabolites had similar biological activity, we tested several related compounds. There was a rigid structural requirement for ethionine in the induction of ovalbumin synthesis. Ovalbumin synthesis produced by ethionine sulfoxide was less extensive than by ethionine (Table 1). In the rat, ethionine is metabolised to ethionine sulfoxide, *N*-acetyethionine and *N*-acetyethionine sulfoxide, but ethionine sulfoxide and *N*-acetyethionine can be converted back to ethionine¹². In the chick the metabolism of *N*-acetyethionine seemed to be different, as it failed to induce ovalbumin synthesis. The sulphone derivative of ethionine and its propyl homologue failed to elicit the synthesis of ovalbumin.

The administration of ethionine plus adenine produced an elevation of up to 10-fold in serum progesterone levels but had no significant effect on the levels of 17- β -oestradiol (Table 2). Progesterone, unlike oestrogen, is incapable of inducing cyto-differentiation of tubular gland cells or ovalbumin synthesis in the unstimulated immature chick oviduct⁴⁻⁶. Ethionine and progesterone are similar in this respect; they produced no pronounced effects on specific protein synthesis without previous oestrogen stimulation³. Furthermore, there is little growth of the oviduct during secondary stimulation with ethionine, another characteristic of the administration of progestins.

Since these determinations were done by radioimmunoassay by a commercial firm (see legend to Table 2), we determined the reproducibility of the individual values. Serum samples were re-submitted for analysis after storage for 1 month at -15°C , under different code numbers. In no case was the variation greater than 2-fold whereas the increment in progesterone level was more than 10-fold. Given the complexity of the assay and the unknown effects of storage, the values obtained may be considered valid.

The mechanism of the elevation of serum progesterone levels by ethionine is obscure, it could be the result of increased synthesis of progesterone or decreased degradation or excretion.

Cholesterol was also determined in the serum of chicks (Table 2). Increase in cholesterol synthesis in the rat is induced by the administration of several carcinogens, such as ethionine, aflatoxin, *N*-2-fluorenylacetamide and 3-methyl-4-dimethyl aminoazobenzene²¹. The effect is thought to be a loss of feedback control of synthesis by dietary cholesterol. These changes in cholesterol synthesis are observed much before the appearance

of any pathological stigmata of tumours. Increased rate of cholesterol synthesis and impairment of its feedback control is also characteristic of a variety of primary and transplantable tumours²¹ and lymphocytic leukaemic cells²². Whether the elevation in the levels of cholesterol which is a precursor of steroid hormones is causal in the elevated levels of progesterone is not known.

Elevation of the progesterone levels by the carcinogen ethionine may have far reaching implications. The importance of endocrine activity in neoplasia is well known²³. Whether this novel effect of ethionine is unique among chemical carcinogens in producing an imbalance in hormone metabolism remains to be determined.

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Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens

SMOOTH muscle tone seems to be primarily regulated by the concentration of free calcium in cytoplasm^{1,2}. Several agents that cause smooth muscle contraction increase the tissue content of cyclic GMP with no significant change or only small reduction of the cyclic AMP concentration³⁻⁷. It has been suggested³⁻⁷ that cyclic GMP may be casually involved in the contractile response of smooth muscle and that this nucleotide may act as a comediator with calcium to promote contraction. Several observations, however, are not consistent with this assumption. Although increases both in tissue tonus^{1,2} and in the cyclic GMP level⁸⁻¹⁰ induced by hormones and neurotransmitters are generally dependent on the presence of extracellular calcium and seem to be secondary to increased influx of calcium into the cytoplasm, the correlation between these two calcium-dependent events is poor in various tissues¹⁰⁻¹⁶. On the basis of such observations, we have suggested that cyclic GMP may act as a negative feedback inhibitor of hormonally stimulated calcium influx into cytoplasm^{8,10,11}. We have studied the effects of various agents on cyclic nucleotide levels in the ductus deferens of the rat, and report here that many smooth muscle relaxants, including sodium nitroprusside (SNP), increase cyclic GMP levels in the ductus deferens.

Although several of the tested agents are more potent in vascular smooth muscle than in tissues of the visceral type of smooth muscle, the ductus deferens was used, since in this tissue the contribution of smooth muscle cells to tissue mass is higher than in most other muscular tissues and the importance of extracellular calcium for hormone effects on cyclic GMP levels is well established⁸⁻¹⁰.

SNP is a potent vasodilator¹⁷ and causes relaxation of the potassium-depolarised ductus deferens (K.D.S., unpublished). Addition of SNP to segments of ductus deferens increased the cyclic GMP level severalfold (Table 1). When

effect on the cyclic GMP level was maximally developed with about 1 mM SNP (see Fig. 1). Cyclic AMP levels were not changed in any of these conditions.

Hydroxylamine increases the cyclic GMP level in rat cerebral cortex²⁰ and caused relaxation of various smooth muscular tissues including ductus deferens (K.D.S., unpublished). In our system, hydroxylamine 0.1 mM increased the cyclic GMP level of the ductus deferens 4-fold, and 20- to 30-fold in the presence and absence of extracellular calcium, respectively; cyclic AMP levels were unchanged (Table 2).

Various other smooth muscle-relaxants and antihypertensives also increased cyclic GMP levels in the rat ductus deferens when they were added in the absence of extracellular calcium (Table 3). Nitroglycerol (0.1 mM) and sodium nitrite (1 mM), agents that have been shown to increase the cyclic GMP content in other tissues^{13,16,21,22} increased the cyclic GMP level of the ductus deferens about 2-fold after 3 min. Furthermore, the antihypertensives diazoxide, hydralazine and minoxidil, the coronary

Fig. 1 Effect of SNP on cyclic nucleotide levels in the ductus deferens. Segments of tissue were preincubated for 30 min in medium without addition of calcium. Sodium nitroprusside was then added for 3 min at the concentrations indicated on the abscissa in *a*. A time course of the response was obtained by adding 0.1 mM sodium nitroprusside for the times indicated on the abscissa in *b*. Data are mean $\pm$ s.e.m., the number of tissue samples per group was 7.

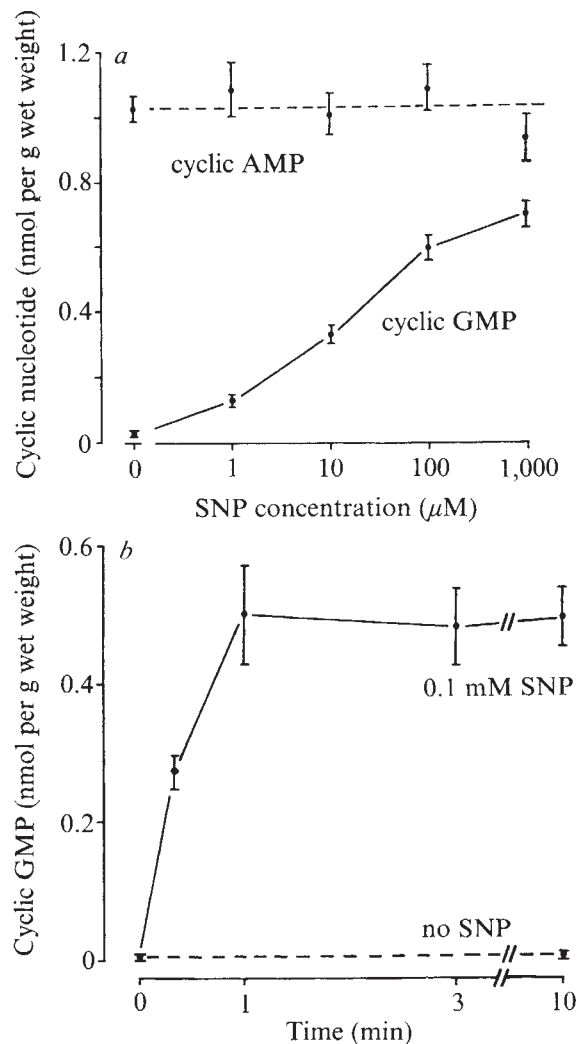


Table 1 Influence of extracellular Ca^{2+} on the effects of SNP and acetylcholine on rat ductus deferens cyclic GMP level

Addition	Cyclic GMP concentration (pmol per g wet weight)	
	2 mM Ca^{2+}	no Ca^{2+}
None (control)	56.4 $\pm$ 5.6 (13)	10.6 $\pm$ 1.5 (15)
Acetylcholine	163 $\pm$ 22.6 (9)	12.7 $\pm$ 2.1 (10)
SNP	585 $\pm$ 79 (7)	596 $\pm$ 39 (7)

Tissue segments were preincubated for 30 min in a balanced salt solution¹⁸ containing 30 μM EDTA and 2 mM or no calcium chloride. Tissue was then transferred to fresh medium containing 2 mM or no Ca^{2+} as above with or without addition of 0.1 mM acetylcholine or 0.1 mM sodium nitroprusside. After 5 min of incubation, tissue was fixed, and cyclic nucleotides were extracted, purified and assayed by established methods¹⁹. Data are mean $\pm$ s.e.m., with the number of samples in parentheses. Cyclic AMP levels were not significantly changed in any of the above conditions.

calcium was omitted from the incubation medium, basal cyclic GMP levels were decreased and the effect of acetylcholine on the cyclic GMP level was abolished, whereas the effect of SNP was still observed. In the absence of extracellular calcium, SNP (0.1 mM) increased the cyclic GMP level about 50-fold within 20 s, and a maximal increase of about 100-fold was observed after 1 min (Fig. 1). Half-maximal stimulation was caused by 10 μM SNP, and the

Table 2 Effect of hydroxylamine on cyclic nucleotide levels in presence and absence of Ca^{2+}

	Concentration (pmol per g wet weight)	
	Cyclic GMP	Cyclic AMP
2 mM Ca^{2+} Control	42.8 $\pm$ 2.9 (7)	914 $\pm$ 34 (8)
Plus hydroxylamine (5 min)	177.7 $\pm$ 25.5 (8)	1002 $\pm$ 91 (8)
no Ca^{2+} Control	9.5 $\pm$ 2.2 (8)	1051 $\pm$ 88 (8)
Plus hydroxylamine (5 min)	195.4 $\pm$ 19.7 (8)	1067 $\pm$ 71 (8)
Plus hydroxylamine (10 min)	260.3 $\pm$ 25.4 (7)	970 $\pm$ 37 (5)

Segments of rat ductus deferens were preincubated for 30 min without addition of calcium. After 5 min incubation with or without 2 mM calcium, hydroxylamine (0.1 mM) was added for 5 to 10 min. Control values were not significantly changed over this period. Values are mean $\pm$ s.e.m.

artery dilators dipyridamole, prenylamine, cinnarizine, lidoflazine, perhexiline, and the methoxy derivative of verapamil (D-600), SKF-525A and chlorpromazine (0.1 mM each) each caused a 2- to 3-fold increase in the cyclic GMP level when added for 3 min to segments of ductus deferens. In contrast, the local anesthetic tetracaine, phenytoin and pentobarbital (0.1 mM each) did not change the cyclic GMP significantly (Table 3), although these agents caused relaxation of the K^+ -depolarised tissue. Cyclic AMP levels were not affected by any of the above compounds.

The mechanism by which these agents increase the cyclic GMP level independently of the presence of calcium is not known. Increased cyclic GMP formation is more likely than reduced degradation of the nucleotide, as the effect of the potent phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine, on the cyclic GMP level in the ductus deferens depended on the presence of calcium whereas the effect on the cyclic AMP level was not affected by calcium⁸. Although several of the above drugs are capable of inhibiting phosphodiesterases in cell-free systems²³, none of the agents changed the cyclic AMP level in the ductus deferens. Furthermore, SNP, sodium nitrite and hydro-

xylamine all stimulate guanylate cyclase from rat ductus deferens (H. Graf and G.S., in preparation).

D-600, prenylamine and several other of the active drugs are known to reduce the responses of smooth muscle and other tissues to hormonal stimuli by their so-called calcium-antagonistic property²⁴⁻²⁶. The 8-bromo derivative of cyclic GMP exogenously applied (10^{-5} M) also reduced the contractile responses of the rat ductus deferens and other tissues to some stimuli and diminished the effect of calcium on the tonus of the K^+ -depolarised ductus deferens (K.D.S., V. Kreye and G.S.). These observations together with our finding that relaxing drugs as well as contraction-producing hormones increase cyclic GMP levels support the hypothesis^{8,10,11} that cyclic GMP may reduce or prevent calcium influx into the cytoplasm of smooth muscle and possibly other cells and may thereby reduce hormone-induced excitation or excitability.

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Table 3 Effect of smooth muscle relaxing drugs on cyclic nucleotide levels

(Experimental series I)	Concentration (pmol per g wet weight)	
	Cyclic GMP	Cyclic AMP
Control	24.6 $\pm$ 4.2 (23)	1052 $\pm$ 35 (25)
Ethanol	29.2 $\pm$ 7.5 (14)	1006 $\pm$ 59 (15)
Nitroglycerol	65.7 $\pm$ 6.9 (14)	1061 $\pm$ 61 (13)
Sodium nitrite	65.1 $\pm$ 7.0 (11)	1001 $\pm$ 81 (5)
D-600	59.9 $\pm$ 6.2 (14)	955 $\pm$ 91 (8)
Cinnarizine	67.4 $\pm$ 14.0 (8)	977 $\pm$ 91 (10)
Lidoflazine	68.6 $\pm$ 17.6 (11)	1020 $\pm$ 78 (11)
Perhexiline	55.2 $\pm$ 14.4 (8)	922 $\pm$ 76 (11)
Minoxidil	84.2 $\pm$ 17.9 (8)	996 $\pm$ 64 (12)
Hydralazine	61.9 $\pm$ 15.7 (10)	1054 $\pm$ 65 (13)
Diazoxide	57.4 $\pm$ 8.3 (10)	1128 $\pm$ 69 (11)
(Experimental series II)		
Control	5.8 $\pm$ 1.1 (8)	1025 $\pm$ 25 (7)
Prenylamine	28.9 $\pm$ 9.5 (7)	954 $\pm$ 31 (8)
Dipyridamole	36.3 $\pm$ 4.4 (7)	972 $\pm$ 50 (6)
Chlorpromazine	29.1 $\pm$ 9.3 (9)	942 $\pm$ 49 (8)
SKF-525 A	22.8 $\pm$ 4.3 (8)	896 $\pm$ 33 (7)
Tetracaine	7.7 $\pm$ 1.5 (9)	970 $\pm$ 61 (9)
Phenytoin	8.3 $\pm$ 3.3 (9)	923 $\pm$ 36 (9)
Pentobarbital	6.7 $\pm$ 2.4 (7)	958 $\pm$ 41 (7)

Segments of ductus deferens were preincubated for 30 min in buffer without addition of calcium. Drugs were then added for 3 min at a concentration of 0.1 mM and sodium nitrite at 1 mM. Ethanol (0.2%, v/v) was used as a solvent of nitroglycerol and perhexiline. Values are mean $\pm$ s.e.m., with number of samples in parentheses.

Trypsin inhibits the action of tetrodotoxin on neurones

EVIDENCE suggests that the molecules responsible for membrane excitability are proteins¹⁻⁴, but they have not been isolated in spite of considerable efforts and their identities remain unknown. Some clues have come from study of the effects of enzymes and group-specific chemicals on appropriate electrophysiological properties of the membrane¹⁻⁴. In certain nerve and muscle cells the voltage-dependent inward currents which generate action potentials may be carried by Na^+ or Ca^{2+} (refs 5-13). Tetrodotoxin (TTX) specifically blocks the voltage-dependent inward Na^+ current in many of these cells¹⁴, including neurones of *Aplysia*¹⁰ and *Helix*¹¹ and is widely used for this reason. It also has the advantage of being a relatively simple

molecule of known atomic structure¹⁴. But TTX has no effect on *Helix* neurones when they are isolated from the ganglia in which they are located¹⁵. The ganglia are first pretreated with trypsin and we wondered whether the lack of TTX-sensitivity in these preparations was due to some action of this enzyme on the TTX receptor. We have now found that exogenous trypsin modifies the TTX-binding properties of the Na channel without affecting its reversal potential or selectivity against Tris⁺ and has no measurable effect on the Ca²⁺ channel. Because trypsin splits polypeptide chains on the carboxyl side of arginine or lysine residues¹⁵, it seems likely that the TTX receptor contains either or both of these amino acids but that the selectivity site does not.

We have developed a simple suction pipette method with which individual nerve cell bodies can be isolated, perfused internally and voltage clamped. This method will be valuable in the study of individual cell membranes of excitable or inexcitable tissues.

We examined neurones from the circumoesophageal ganglia of *Helix aspersa* using a suction pipette with an internal diameter of 15–30 μm (Fig. 1), filled with a solution of 50 mM K aspartate and 55 mM KH₂PO₄ adjusted to

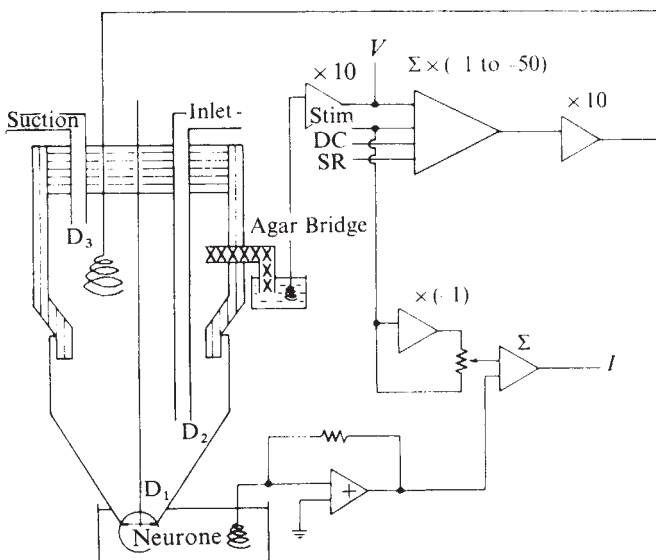


Fig. 1 Schematic of experimental setup. D₁ = 25 μm , D₂ = 500 μm , and D₃ = 2 mm. SR, series resistance compensation. Σ is the summing amplifier used to offset the shunt resistance.

pH 7.4 with 28 mM Tris⁺ base¹⁶. The extracellular solution bathing the neurones had the following composition: 75 mM NaCl; 5 mM KCl; 10 mM CaCl₂; 15 mM MgCl₂, and 5 mM Tris-HCl, pH 7.6. The neurones were exposed by stripping off the capsule of the ganglion with jeweller's forceps. A neurone with a diameter ranging from 80 to 250 μm was sucked into the tip of the pipette and isolated by rending it free from neighbouring neurones. A platinised platinum wire (5 μm tip) was then advanced into the neurone. It had a resistance of $3 \times 10^4 \Omega$ and was connected through an agar salt bridge and Ag/AgCl wire to the voltage amplifier (Fig. 1). A reference for the voltage was also provided by a salt bridge connected to the bath, but this is not shown in Fig. 1. In these conditions and at a temperature of 18 °C we measured resting potentials of -60 mV (after corrections for measured liquid junction potentials), input resistances of 15–20 M Ω , and action potentials with overshoots of 20–30 mV for periods averaging

6 h. Similar values were obtained when two 3 M KCl-filled micropipettes, one for passing current and the other for recording voltage, were inserted into the same neurone. These measurements indicate healthy neurones and we presume that the soma healed over at the site where it had been pulled free from its axon much the same way as cut cardiac muscle heals over.

The voltage clamp circuit included series resistance compensation¹⁷ and a circuit for correcting the offset currents passing across the shunt resistance of 50 and 100 M Ω (ref. 18) that isolated the cell body from the extracellular solution (Fig. 1).

The voltage clamp currents in the control solutions were elicited by voltage steps from holding potentials of -60 mV and had voltage-dependent Na⁺, Ca²⁺ and K⁺ components (Fig. 2a). They were separated from each other as follows.

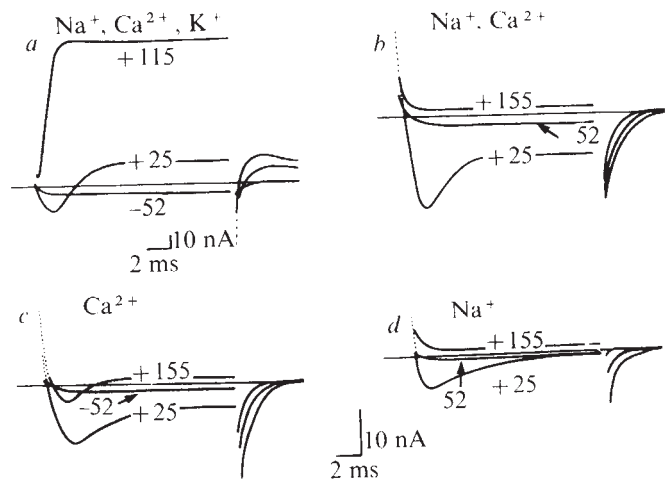


Fig. 2 Voltage clamp currents and their separation in *Helix* neurones. Holding potential, V_H = -60 mV. Voltage clamp potentials indicated above respective currents. Note the differences in current calibration between (a) and (b), (c) and (d). a, Neurone perfused internally with control solution (see text). External solution contained CsCl. The voltage-dependent currents are due to Na⁺, Ca²⁺ and K⁺. The small depolarising step produces sustained inward current. The current tails are outward for the larger voltage steps. b, c and d, Current traces from a different neurone. b, After 20 min of internal perfusion with Cs aspartate, the voltage-dependent outward K⁺ current is abolished. The remaining voltage-dependent currents are due to Na⁺ and Ca²⁺. Early current is outward at +155 mV and the persistent outward current is attributed to leakage. At +25 mV there is an early transient inward current and a later smaller persistent inward current. The tail currents are now inward at all steps and appear to recover more rapidly. c, Ten minutes after 3×10^{-5} M TTX was added to external solution Ca²⁺ current is separated. Notice early inward current at +155 mV, indicating that E_{Ca} is more positive than this value. Inward current at +25 mV is reduced by about 30%. The Ca²⁺ current is abolished by verapamil or Co²⁺. d, Same neurone as (b) and (c), 30 min after recovery from TTX and 5 min after addition of 5×10^{-5} M verapamil to the external solution. The Na⁺ current is separated. The early current is outward at +155 mV, shows little inactivation at -52 mV, but inactivates almost completely at +25 mV. The Na⁺ tail currents are smaller than the Ca²⁺ tail currents. The Na⁺ current is blocked by TTX and substitution of Tris for Na⁺ in the external solution.

The K⁺ current was blocked by substituting 110 mM Cs aspartate for the potassium salts in the control internal perfusion solution (Fig. 2b). We also substituted Cs⁺ for K⁺ in the extracellular solution. Addition of TEA to the internal or external media had no further effects. We used TTX (Sigma or Sankyo) to block the Na⁺ current. The action of TTX was dose dependent and reversible. TTX in doses of 3×10^{-5} M reduced the inward current by an average of 30% in 40 neurones (Fig. 2c). Similar doses were also effective in *Aplysia* neurones¹⁹. Replacement of Na⁺ in the external solution with Tris⁺ had a similar effect to TTX

in 20 neurones. Thus, 30% of the inward current was carried by Na^+ . This Na^+ current was isolated after blocking Ca^{2+} current with verapamil (Knoll) in doses of $1\text{--}5 \times 10^{-5}$ M or by substituting Co^{2+} for Ca^{2+} (Fig. 2d). The Na^+ current has a transient peak and then inactivates although the time course may vary among different neurones.

After TTX or Tris^+ substitution for Na^+ , the inward current was carried by Ca^{2+} (Fig. 2c). A component of the Ca^{2+} current elicited by small depolarisations showed little or no inactivation as previously reported¹⁹. At larger voltages the inward Ca^{2+} current also had a transient peak. The calcium was increased or decreased when extracellular calcium was doubled or halved, and reduced or abolished by when Co^{2+} was substituted for Ca^{2+} or verapamil was added. In TTX, the Ca^{2+} current was unchanged when Tris^+ was substituted for Na^+ . Substitution of methanesulphonate ion for Cl^- had no effect on any of these currents.

Having established the presence of a TTX-sensitive Na^+ current, we tested the effects of trypsin (type III, Sigma) on this current in 20 neurones. The outward K^+ and inward Ca^{2+} currents were blocked and the inward Na^+ currents elicited by 20-mV voltage steps from -60 to $+160$ mV were recorded. Such Na^+ currents could be blocked by TTX or substitution of Tris^+ for Na^+ (Fig. 3a). Neurones with the inward Na^+ current separated in this way were then exposed to 0.1% trypsin for about 2–4 min. Exposure to these doses of trypsin for this period increased membrane resistance slightly but did not affect the magnitude of the inward currents or their reversal potentials. In some instances, the time course was slightly altered. TTX now had no effect on the Na^+ current (Fig. 3d). But substitution of Tris^+ for Na^+ still blocked the inward current (Fig. 3e).

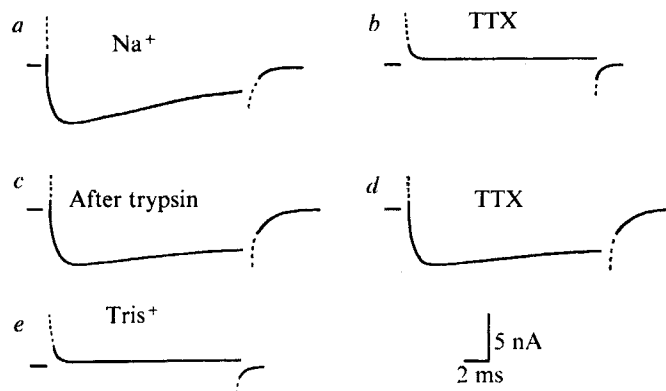


Fig. 3 Na^+ current in a *Helix* neurone, its blockage by TTX and the effects of trypsin on the Na^+ current and the TTX blockage. $V_H = -60$ mV. All currents elicited by voltage steps to $+25$ mV. Internal perfusion with Cs aspartate, external solution contains CsCl (see text). Ca^{2+} currents blocked by verapamil. *a*, Control Na^+ current. *b*, After 5 min in 3×10^{-5} M TTX. Only the outward leakage current persists. *c*, After recovery in TTX-free solution for 30 min and exposure to 0.1% trypsin for 3 min. The magnitude of the inward current is unchanged but inactivates more slowly. This is not, however, a consistent finding. *d*, After 10 min exposure to 1.5×10^{-4} M TTX in trypsin-free solution. TTX no longer blocks Na^+ current. *e*, Substitution of Tris^+ for Na^+ in the external solution eliminates the Na^+ current leaving a small outward leakage current.

To examine whether trypsin acted on the Ca^{2+} current, we separated it from the Na^+ and K^+ currents and compared the inward currents before and after enzyme treatment. Equivalent exposures which abolished TTX-sensitivity had no effect on either calcium currents or their blockage by verapamil or Co^{2+} . Exposures of 10 min, however, reduced membrane resistance and Ca^{2+} currents as well as the TTX-insensitive Na^+ current.

Trypsin destroys the TTX-sensitivity of the neuronal

membrane without affecting the magnitude and time course of the inward currents. This accounts for the absence of TTX sensitivity in snail neurones¹³ pretreated with this enzyme. The action is specific since Na^+ current persists and the Ca^{2+} current is unaffected. The external application of trypsin to lobster giant axon¹, or α -chymotrypsin to squid giant axon⁴, was also without effect on the membrane's electrical properties although another enzyme, Pronase, when applied internally to squid giant axon, removes Na^+ inactivation selectively³. If the guanidinium group of the TTX molecule blocks the Na^+ channel at the selectivity filter²⁰, the persistence of a TTX-insensitive inward Na^+ current with an unchanged reversal potential suggests that the selectivity filter has not been affected by trypsin. Thus, trypsin may act on the part of the channel occupied by the remainder of the TTX molecule and this portion probably contains lysine and/or arginine residues. It is also interesting that modifications of TTX at carbon positions 4 and 9, which are close to the guanidinium group, greatly reduce the potency of the toxin²¹. The lack of TTX sensitivity of some excitable cells, such as puffer fish neurones²² and denervated skeletal muscle²³ cells, may have a similar basis.

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Formation *in vivo* of volatile N-nitrosamines in man after ingestion of cooked bacon and spinach

N-NITROSAMINES, which are thought to be causally related to human cancer¹, have been found in $\mu\text{g kg}^{-1}$ concentrations in polluted air^{2,3}, in water⁴ and in tobacco products⁵. Foodstuffs such as cooked bacon, preserved with nitrite, have long been known to contain $\mu\text{g kg}^{-1}$ amounts of volatile N-nitrosamines formed during cooking⁶. *In vivo* formation of N-nitrosamines, after ingestion of suitable amine precursors and nitrite, has been demonstrated in

laboratory animals⁷. *In vivo* formation in human subjects with hypoacidity, gavaged with relatively massive amounts of diphenylamine plus nitrite, has been demonstrated through detection of *N*-nitrosodiphenylamine in the stomach contents⁸. *In vivo* nitrosation after ingestion of conventional foods has not been demonstrated so far, either in animals or man. We now report *in vivo* formation of volatile *N*-nitrosamines in man after ingestion of a midday

meal consisting of a bacon, spinach and tomato sandwich and beer.

Blood (20 ml) was taken on the day before the test. On the day of the test, 20 ml of blood was taken 50 min before the noon meal. Lunch consisted of 310 g of spinach, 170 g of cooked bacon, 200 g of tomatoes, 120 g of bread and 460 g of beer. One per cent of the total lunch was analysed for volatile *N*-nitrosamines. Blood samples (20 ml) were taken 35, 65, 162 and 220 min after the meal. A final control sample was taken at 1,300 min. Samples were poured immediately into liquid nitrogen. Less than 20 s elapsed between the end of collection and freezing in liquid nitrogen. Mineral oil was added to the whole blood, which was analysed for volatile *N*-nitrosamines by standard procedures⁹. Control experiments were performed by recovering *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA) and *N*-nitrosopyrrolidine (NPYRR) at the $0.4 \mu\text{g kg}^{-1}$ level (0.4 p.p.b.) from whole human blood (obtained from a blood bank). The blood from the bank contained no volatile *N*-nitrosamines.

The meal contained preformed NDMA and NPYRR. The total amount of preformed NDMA ingested was 1,600 ng. Identification of NDMA and NDEA was confirmed using both TEA-GLC and TEA-HPLC. Figure 1 shows the chromatographic traces of the blood sample taken 162 min after the meal. The TEA-GLC trace shows only two peaks, those at the retention time of NDMA and NDEA. The TEA-HPLC trace shows three peaks, two eluting at the same retention time as NDMA and NDEA (note that the peak order is reversed). Furthermore, the quantities of NDMA and NDEA are identical for both the GLC and HPLC systems. As a further confirmatory step, the NDMA and NDEA peaks eluting on the HPLC were

Fig. 1 *a*, High-pressure-liquid-chromatogram for a 10- μl extract of whole human blood. The TEA-HPLC was constructed¹⁰ by combining an injector (Waters Associates, model UK6), with a high-pressure pump (Waters Associates, model 6000A) and a thermal energy analyser (Thermo Electron, model TEA-HPLC 502). A μm Porasil column was used with a 5% acetone-95% 2,3,4-trimethylpentane solvent system at a flow rate of 2 ml min^{-1} . TEA attenuation was $\times 2$. *b*, Gas-liquid chromatogram for a 10- μl extract of whole human blood. A single-column isothermal gas chromatograph was constructed¹¹ from 14 feet of stainless steel tubing (outer diameter 1/8 inch), packed with 10% Carbowax 20 M on Chromasorb W, 80-100 mesh. The column temperature was 180°C , with Ar carrier gas at a flow of 25 ml min^{-1} . A thermal energy analyser (Thermo Electron, model TEA-502) was used as the detector. TEA attenuation was $\times 2$.

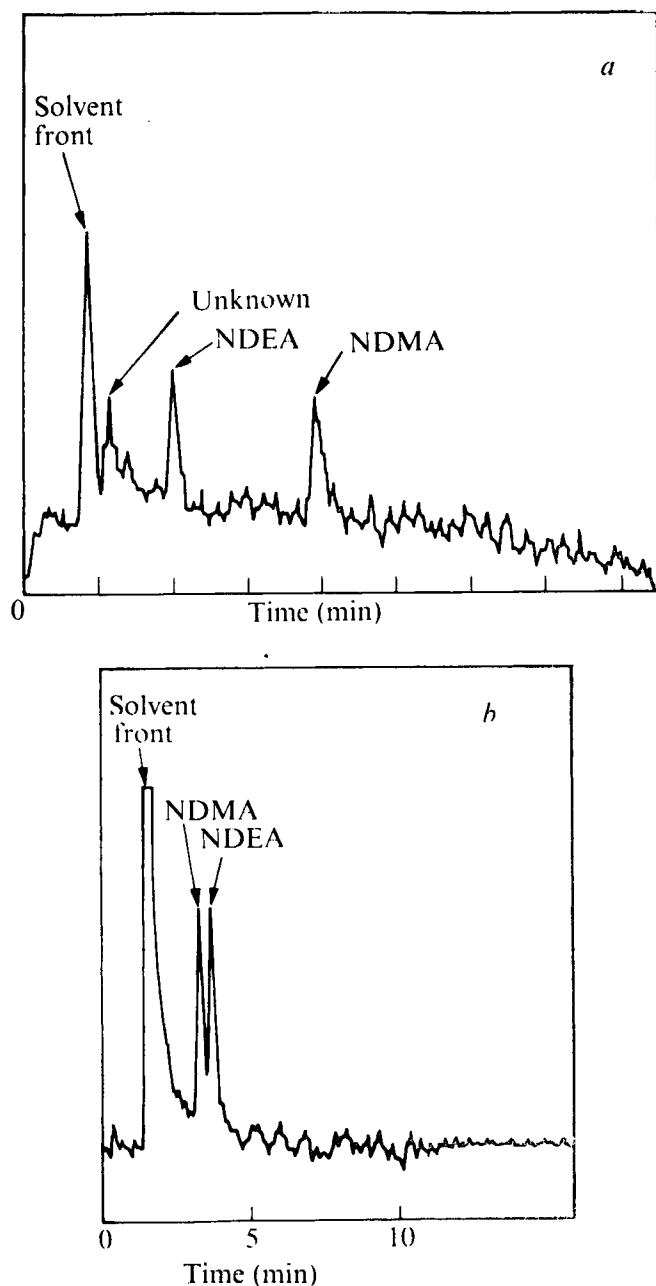
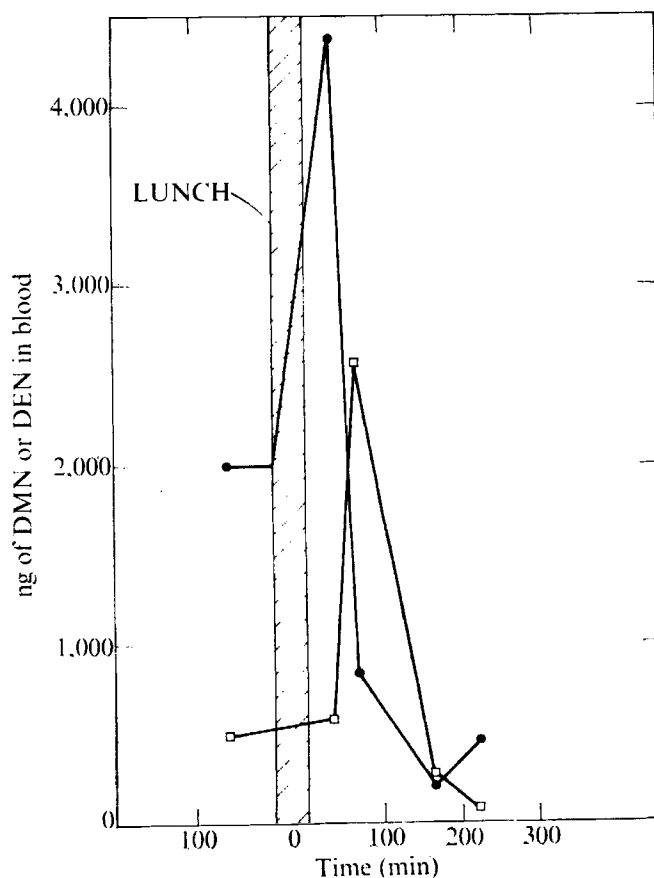


Fig. 2 *In vivo* nitrosation in man after ingestion of bacon, spinach, tomato and beer. ●, NDMA; □, NDEA.



isolated and reconstituted and then injected on to the GLC. The peak corresponding to NDMA on the HPLC gave only a single peak on the GLC, corresponding to NDMA. The same was true for NDEA. Given the selectivity of the TEA, evidence for the identity of volatile *N*-nitrosamines based on parallel TEA-GLC and TEA-HPLC procedures can be taken as confirmatory^{10,11}.

The amount of NDMA and NDEA present as a function of time in the blood (assuming total body blood is 5,640 ml) is shown in Fig. 2. Before the meal the blood contained 2,000 ng of NDMA and 510 ng of NDEA; 35 min after the meal NDMA had increased to 4,350 ng and NDEA to 570 ng, and 65 min after the meal, NDMA had fallen to 860 ng while NDEA had increased to 2,600 ng. At 162 min, both had decreased below the levels observed before the meal. At 1,300 min there was 760 ng of NDMA in the blood; NDEA was not present. NPYRR was not detected in the blood either before or after the meal.

These amounts of NDMA in human blood imply that the compound was being formed *in vivo* in quantities greater than those present before the meal. The total amount of NDMA in the body may be considerably in excess of that reported here because we assumed that all NDMA was taken up in the blood alone—an appreciable amount may have been exhaled or absorbed in various tissues. NDEA was not found in the food, but it was formed *in vivo* after the meal. It is interesting that the content of NDEA was maximal after the concentration of NDMA had reached its maximum. Our experiments were carried out three times, giving similar results for NDMA, although the actual measurements differed in each case. NDEA was detected in only one of the three experiments. The source of the background NDMA in the blood is unknown; no background was observed if the volunteer had eaten a bland low nitrate-nitrite, high ascorbate diet for the previous 24 h.

The experiments reported here, although simple, demonstrate conclusively that *N*-nitrosamines can be formed *in vivo* in man after ingestion of conventional foodstuffs.

The ability routinely to detect nitrosation *in vivo* should make possible realistic tests of putative environmental carcinogens.

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Ferritinaemia in cancer

SMALL amounts of ferritin are found in serum in normal and pathological states^{1,2}, usually at levels directly related to the amount of tissue storage iron^{3,4}. This relationship, however, does not hold in many cancers where grossly elevated levels can occur without a corresponding increase in iron stores^{5–9}. These latter findings suggest a possible use of ferritinaemia in cancer diagnosis, but the value of this test has not been clearly established. Such use is complicated by the multiple potential sources of the serum ferritin which could affect its type as well as its amount. Much of the ferritin in cancer sera may reflect increased iron stores arising from chronic anaemia or from transfusions in treated patients. Some may also come from nonspecific tissue damage. The high levels of ferritin synthesis in some cancer cells¹⁰ and the presence in serum of iso-ferritins characteristic of some cancer and foetal tissues^{11,12}, however, suggest that much of the ferritin may come directly from cancer cells. If so, selective quantitation of these iso-ferritins may provide specificity in possible serodiagnosis. The multiple iso-ferritins found in most human tissues seem to be hybrid molecules composed of different proportions of two subunit types¹³. One type predominates in liver and spleen iso-ferritins, and the other in the more acidic iso-ferritins in heart, tumours and HeLa cells¹⁴. Different iso-ferritin populations can be distinguished immunologically, apparently on the basis of their subunit composition^{14,15}. Ferritin is usually assayed by immunological methods, with crystalline liver or spleen ferritins as immunising antigens and reference standards. These ferritins consist almost entirely of the liver-type subunit¹⁵. Consequently, most assays based on these ferritins have only a low reactivity for the more acidic 'carcinofoetal' iso-ferritins which may contain less than 20% of the liver-type subunit^{14,15}. We have, therefore, tested a variety of cancer sera and compared the apparent levels of ferritin given by a standard 'liver-type' assay with levels given by an assay whose specificity is directed against the more acidic iso-ferritins¹⁵. We report here preliminary evidence that the latter type of assay may significantly improve the usefulness of ferritinaemia as a tumour marker.

Serum samples from untreated patients with various histologically confirmed malignancies were provided by Dr Elliot Alpert, Massachusetts General Hospital, Boston, Mass. The leukaemic sera were obtained from New England Medical Center, Boston. The samples were not graded according to the stage of disease or other pathological disorders. The levels of ferritin in these sera were estimated by two radioimmunoassays (RIA). One RIA reacts preferentially with liver-type iso-ferritins, the other with the more acidic iso-ferritins characteristic of heart and HeLa cells¹⁵. Antibodies for the 'liver-type' RIA were raised in rabbits against crystalline human liver ferritin consisting of approximately 90% of the liver-type subunit. Antibodies for the 'HeLa-type' RIA were developed against HeLa ferritin (>80% heart-type subunit). Before use, the anti-HeLa antiserum was absorbed with a natural apoferritin preparation (>95% liver-type subunit) to restrict its specificity to sites determined by the heart-type subunit¹⁵.

The apparent level of the serum ferritin from 8 out of 12 different cancers was substantially higher when estimated with the 'HeLa-type' than with the 'liver-type' assay (Table 1). In most cases, the ratio of the values given by the two assays (HeLa-liver) was between 2 and 5. These ratios are markedly different from those obtained in non-malignant conditions, such as iron overload, where the ratio was less than 0.1. This differential indicates that the ferritin in these cancer sera contained substantially higher proportions of the heart-type subunit than the serum ferritins from iron overload. This conclusion must also apply even in cases where the apparent level given by the 'HeLa-type' RIA was slightly lower than that given by the 'liver-type' RIA (for example, cases 4, 21).

Table 1 Serum ferritin levels (ng ml⁻¹)

Case no.	Diagnosis	Liver-type	HeLa-type	Ratio HeLa/liver
1	Normal	77 ± 48	ND	—
2	Hemochromatosis	5000	190	0.04
3	Hemochromatosis	950	ND	—
4	Teratoblastoma	1550	1000	0.6
5	Glioma	130	ND	—
6	Ovarian carcinoma	62	430	7.1
7	Breast cancer	205	500	2.4
8	Breast cancer	330	800	2.4
9	Breast cancer	190	350	1.8
10	Breast cancer	50	140	2.8
11	Pancreatic carcinoma	600	1900	3.1
12	Pancreatic carcinoma	320	600	1.9
13	Pancreatic carcinoma	460	500	1.1
14	Hepatoma	560	850	1.5
15	Gall bladder carcinoma	550	2700	5.0
16	Colon carcinoma	220	1500	6.7
17	Stomach carcinoma	750	1750	2.4
18	Stomach carcinoma	118	235	2.0
19	Stomach carcinoma	550	1050	1.9
20	Lung carcinoma	630	1750	2.8
21	Multiple myeloma	2630	1350	0.5
22	Acute lymphoblastic leukaemia	3800	165	0.04
23	Acute lymphoblastic leukaemia	34670	130	0.004
24	Acute myeloblastic leukaemia	1675	ND	—
25	Acute myeloblastic leukaemia	1866	135	0.07
26	Pancreatitis	1170	740	0.6

Values given as ND were below the minimum detectable level at 100 ng ml⁻¹ in the 'HeLa-type' assay. The difference in sensitivities of the two assays reflects our inability to iodinate HeLa ferritin to the same extent as liver ferritin.

Although the differential in serum ferritin levels given by the two assays holds in most cancers examined here, some, such as the leukaemias, did not conform to this general pattern. The very low HeLa–liver ratio in these samples could reflect liver-type isoferritins in the cancer cells or increased iron stores due to deficient erythropoiesis. High levels of the more acidic isoferritins have been found in untreated leukaemias⁴.

The immunological differences among human isoferritins might be of value in obtaining information about serum ferritin phenotypes in various pathological states. Our results indicate that the 'HeLa-type' RIA might not only add a key element of specificity for assessing ferritinaemia in many cancer sera but might also detect abnormally high levels which might not be evident with the 'liver-type' assay (for example, cases 6, 7, 9, 16). If the more acidic isoferritins reflect high levels of secretion, rather than cell damage, their detection may prove useful in early diagnosis. It should be noted, however, that elevated levels of these isoferritins in sera are unlikely to be entirely specific for cancer. These isoferritins occur in small amounts in heart, kidney and pancreas¹⁶ and their level in serum may increase in conditions such as myocardial infarction^{5,8} and pancreatitis (case 26). More thorough studies are required to extend our preliminary observations and to correlate the type and amount of serum ferritin in cancer and other pathological states throughout the course of the disease.

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Phosphorus nuclear magnetic resonance of perfused working rat hearts

THERE are serious limitations to techniques used to correlate biochemical parameters with function in metabolically active tissues. For the perfused heart such techniques include measurement of surface NADH fluorescence², comparison of coronary arterial–venous composition, and biochemical analysis of intracellular metabolites from biopsy specimens. Although the latter approach has yielded critical information, it is limited because local injury prevents accurate interpretation of mechanical function. Recently ³¹P nuclear magnetic resonance (NMR) has been used to analyse excised muscles and their acid extracts for intracellular concentrations of the sugar phosphates, glycolytic intermediates, free orthophosphates, phosphocreatine, ADP and ATP. In addition, the pH-dependent chemical shift of the orthophosphate resonance has been titrated³ and used to determine the intracellular pH of intact muscles⁴. These muscles were neither perfused nor functioning, but we report here the assessment by ³¹P NMR of the myocardial energy status in the working, perfused rat heart subjected to various degrees of ischaemia.

Albino Sprague–Dawley rats (200 g) were injected with 1,000 U of heparin and anaesthetised with 20 mg of pentobarbital. The hearts were excised, washed with cold perfusate and, within 3 min, perfused by means of a cannula inserted into the aorta

and positioned above the aortic valve. The cannula was attached to a column 107 cm high. Left ventricular pressure was monitored by means of a catheter, 100 cm long, inserted through the left atrial appendage and mitral valve, and connected to a Statham P23Db strain gauge transducer and a Brush 220 recorder. The perfusate was maintained at 30 °C and was bubbled continuously with pure oxygen. It contained 145 mM NaCl, 6 mM KCl, 1.3 mM CaCl₂, 0.4 mM MgSO₄, 16.7 mM glucose, 20 mM Tris-Cl (pH 7.4) and 1% Dextran 70.

³¹P NMR spectra were obtained on a Nicolet TT 23 pulsed Fourier transform spectrometer interfaced to a Varian V 4412 probe. The heart was contained in a 12-mm NMR tube. Field-frequency control was obtained by locking on the deuter-

the resulting ³¹P spectrum is shown in Fig. 3d. Obviously, the phosphocreatine and ATP resonances increased above the ischaemic levels while the inorganic phosphate resonance decreased. Apparently, the total intensity of the phosphorus resonance decreased with time, presumably due to leakage of inorganic phosphate from the heart into the perfusate during ischaemia. The pH of the tissue was monitored by the chemical shift of inorganic phosphate. The results for the heart in various conditions are shown in Table 1. Intracellular pH decreased by 1.2 pH units during partial ischaemia and by a further 0.5 pH unit during total ischaemia.

In several experiments (spectra not shown) the linewidth of the P_i resonance tended to be greater in fully perfused heart

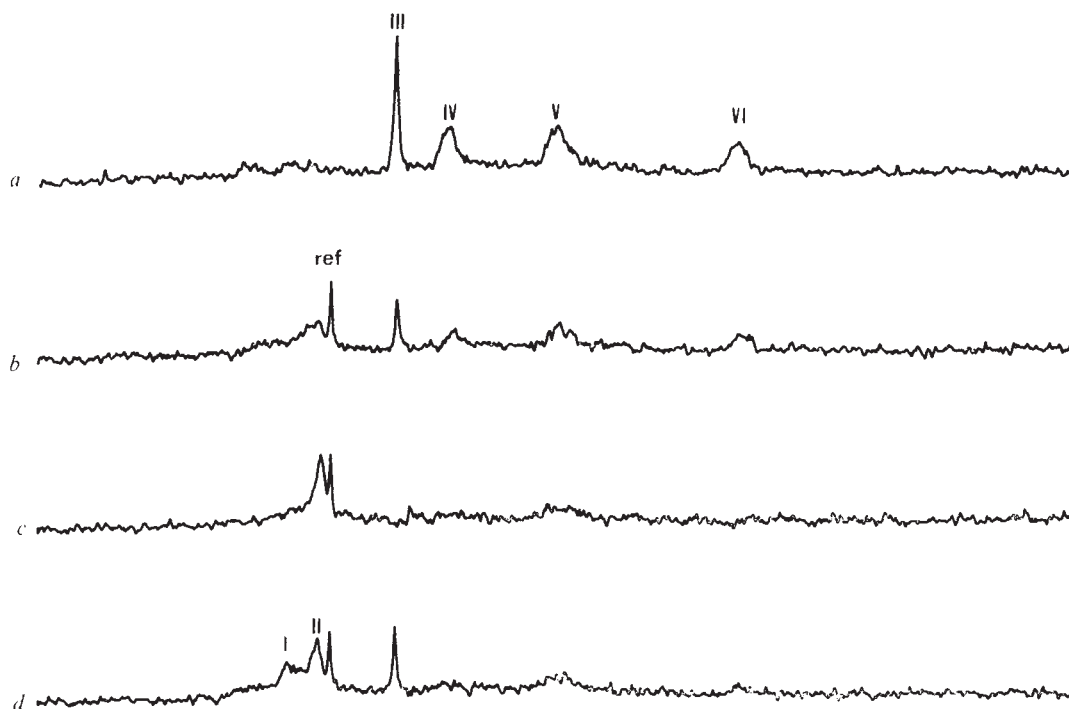


Fig. 1 ³¹P NMR Spectra of working perfused rat hearts (40.5 MHz). *a*, Normal perfusion pressure of 107 cm H₂O. *b*, partial ischaemia, at a perfusion pressure of 43 cm H₂O. *c*, Total ischaemia with aortic cross-clamp. *d*, Reperfusion at 107 cm H₂O. Peak assignments: I, sugar phosphates; II, orthophosphate; III, phosphocreatine; IV, γ ATP; V, α ATP; VI, β ATP.

ium signal from a 3-mm glass tube, containing pure D₂O, which was slipped into the NMR tube alongside the pulsing heart. Where desired, an external reference signal was obtained from a 1-mm capillary containing 0.2 M H₃PO₄ in 15% HClO₄. Typically, data was obtained using an 8K data table, a 2,000-Hz spectral width, a 2.0-s acquisition time and 1,000 transients. Probe temperature was maintained at 30 °C.

Figure 1a shows the ³¹P spectrum of a beating perfused rat heart in optimum conditions of perfusion. Assignments are taken from earlier work on excised muscle^{2,3}. The active muscle is characterised by prominent resonances from the three phosphates of ATP and the phosphate group of phosphocreatine and only weak resonances from inorganic phosphate and sugar phosphates. When partial ischaemia was produced by reducing the perfusion column from 107 to 42 cm H₂O, the heart rate declined from 214 to 178 beats per min and left ventricular pressure declined as expected (Fig. 1b). The intensities of the ATP and phosphocreatine resonances decreased markedly relative to that of inorganic phosphate. Figure 1c shows the effect of 25 min of total ischaemia produced by cross clamping the aortic cannula. During total ischaemia the heart stopped pulsing. Neither phosphocreatine nor ATP resonances were detectable but the inorganic phosphate resonance increased markedly. With reperfusion the heart started to beat again, and

than in either partially or totally ischaemic heart. This suggests that pH gradients existing in oxygenated heart are dissipated in ischaemia. During reperfusion of ischaemic heart a small signal appeared in the region expected for sugar phosphates. This may reflect a buildup of glycolytic intermediates before some critically inhibited enzymatic step.

Table 1 Intracellular pH measured by ³¹P NMR

Condition	Perfusion pressure (cm H ₂ O)	Chemical shift (p.p.m. from H ₃ PO ₄ -HClO ₄ reference)	pH
Normal	107	2.04	7.4
Partial Ischaemia	43	0.86	6.2
Total Ischaemia	0	0.46	5.7
Reperfusion	107	0.68	6.1

Thus we have explored both the energy status of a tissue and its intracellular pH. Biochemical data were collected in steady-state conditions and correlated well with changes in mechanical function. With minor modifications our method is applicable

to liver, kidney and other perfused tissues, and we believe that it represents a useful new technique.

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Cloning and expression of the flagellar hook gene on hybrid plasmids in minicells

SPECIFIC *Escherichia coli* DNA fragments carrying flagellar genes have been cloned on λ vehicles and gene expression has been studied in λ -infected ultraviolet irradiated-cells^{1,2}. This work has led to the identification of several gene products involved in flagellar function. But, the structural gene for the hook subunit protein, which is one of the major flagellar components has not been identified. This gene could have been missed if it was not part of the DNA that was cloned, if it was cleaved during endonuclease treatment or if it was transcribed at such a low level that the product could not be detected in ultraviolet-irradiated cells. We report here another approach which avoids these

difficulties and has enabled us to identify the region of the genome which carries genes involved in the synthesis of flagellar hook and basal structures. This result is interesting for two reasons. First, it demonstrates the general applicability of cloning techniques to the identification of any gene product in *E. coli*. Second, it focuses attention on a hitherto poorly defined group of genes involved in flagellar structure and function.

Various proteins are associated with *E. coli* flagellar structure and function. The flagellar organelle seems to be composed of three sections: the filament, the hook and the basal structure³⁻⁵. The filament is made up of a single polypeptide subunit called flagellin with an apparent molecular weight of 53,000 (ref. 6). The hook is also composed of a single polypeptide subunit with a molecular weight of 42,000. The hook subunit is clearly distinguished from flagellin by its specific reaction with antihook subunit antiserum⁷. The flagellar filament rotates, and the hook may act as a flexible 'universal joint' connecting the filament to the basal structure. The basal structure is complex and composed of at least nine different polypeptides⁸. Other proteins involved in flagellar formation and function are localised in the cell membrane and cytoplasm^{2,9}.

The genes responsible for flagellar formation have been defined by genetic complementation tests, and more than 20 of them were found to map in three regions of the *E. coli* chromosome (Fig. 1). Some of these genes have regulatory functions. Thus strains that carried mutations in the *fla* gene did not synthesis hook subunits or other flagellar component proteins^{7,10}; whereas strains that carried mutations in the *flaE* gene seemed to over-produce the hook subunit protein and formed polyhooks. The *flaE* gene is not responsible for the synthesis of the hook subunit protein^{7,10}. Mutants that have altered hook subunit proteins have not been found. To find the structural gene, it was necessary to use the plasmid-*E. coli* hybrid system and study the expression of genes carried by plasmids that were segregated into minicells.

Clark and Carbon^{11,12} prepared a colony bank of strains

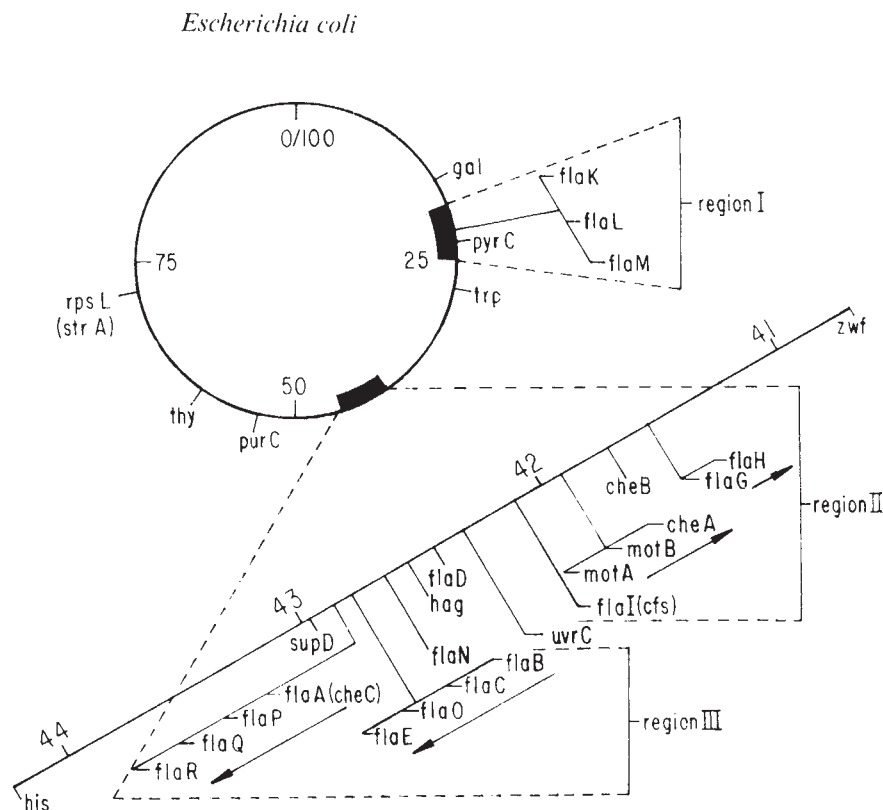


Fig. 1 The map positions of genes controlling flagellar structure and function in *E. coli*. The gene designations have been described before^{2,11,7}. *fla* refers to genes that include mutation which result in strains that have no apparent flagellar structure; *hag* is the structural gene for the major subunit protein of the flagellar filament; *mot* refers to mutations that result in paralysed flagella; *che* includes strains that do not respond to chemotactic signals; *cfs* refers to a regulatory locus controlling the sensitivity of flagellar formation to cyclic AMP (ref. 7).

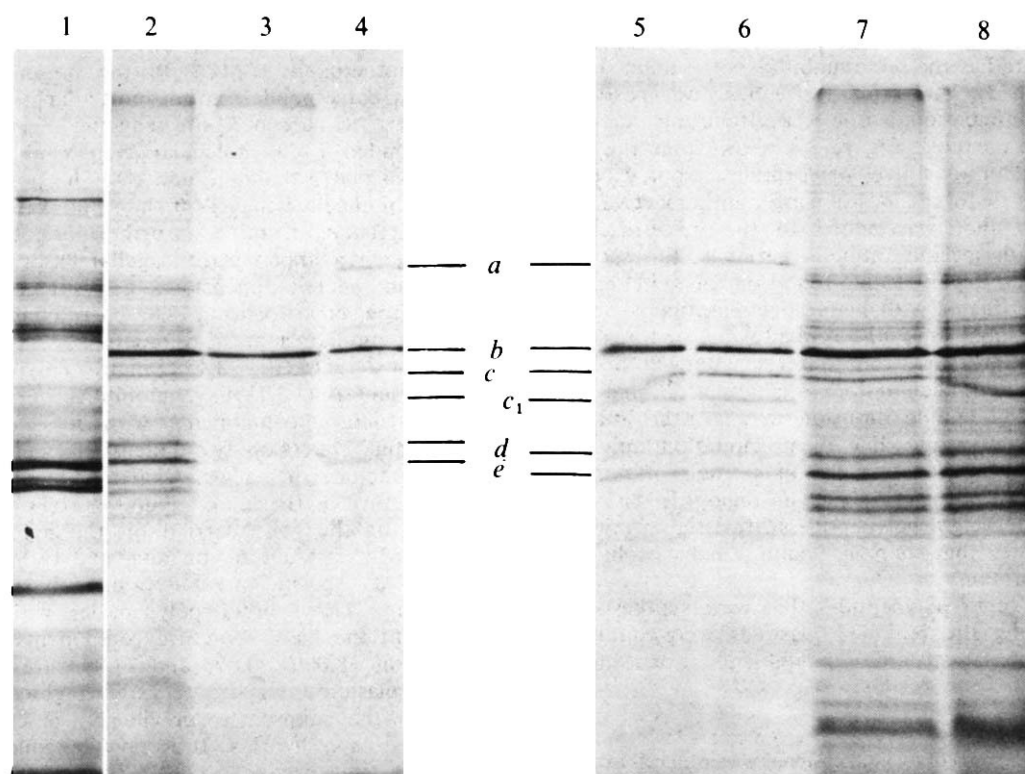


Fig. 2 Autoradiograms of SDS-acrylamide electrophoresis gels of minicells carrying hybrid plasmids. The plasmids were transferred by conjugation into minicell producing strain P678-54 (obtained from R. Sparks, University of California, San Diego). All plasmid-carrying strains were from the Clarke and Carbon collection¹². Minicells were collected after the parent strain had been grown for 14 h in L broth and thymine ($15 \mu\text{g ml}^{-1}$) at 37°C . The minicells were purified by differential centrifugation and sucrose gradients¹³. They were pre-incubated in M-9 minimal medium with glycerol supplemented with 19 amino acids ($4 \mu\text{g ml}^{-1}$); no methionine was added. After incubation at 37°C for 20 min, ^{35}S -methionine ($20 \mu\text{Ci ml}^{-1}$, 350 Ci mmol^{-1}) was added and incubation continued for 30 min. The minicells were collected and lysed in buffer containing 3% SDS and 0.5 M βME . Samples were heated to 100°C for 2 min. The electrophoresis on 12 1/2% acrylamide gels has been described before². In samples 5 and 8, βME was omitted. For antibody precipitation, the minicells were lysed by treatment with EDTA and lysozyme⁸ and then sonicated for 2 min. The debris was removed by centrifugation for 45 min. The supernatant was dialysed, lyophilised and reconstituted, and nonradioactive hook subunit protein was added as carrier. Anti-hook subunit antibody 926 (ref. 7) was used to precipitate the protein at equivalence. The precipitate was washed twice with cold buffered saline and resuspended in lysis buffer. Sample 1, minicells with plasmid pLC 7-18 carrying *flaD*; sample 2, minicells with plasmid pLC 11-15 carrying *flaK*, *flaL* and *flaM*; sample 3, pellet after antibody precipitation of extract of minicells carrying pLC 11-15; samples 4 and 6, hook and basal structure components prepared as previously described⁶; samples 5, same as 4 and 6, except that no βME was added; sample 8, same as 2 and 7, except that no βME was added. The letters in the centre refer to the position of some of the components of the basal structure that are found after the purified hook-basal structure⁸ is dissociated. *a*, Component with apparent molecular weight of 60,000; *b*, hook subunit protein, *c* and *c*₁, positions assumed by component in the presence and absence of added βME ; *d*, component with apparent molecular weight of 30,000; *e*, 27,000 molecular weight component.

with hybrid *E. coli*-colicinogenic factor E_1 (Col EI) plasmids. Each hybrid carried a randomly selected fragment of *E. coli* DNA so that the bank, consisting of more than 2,000 clones, carried within it all the *E. coli* genes. Furthermore, each strain had an F factor which allowed the transfer of the hybrid plasmid. These strains were tested by mating into various *fla*⁻ recipients on motility plates. The clones that were able to transfer motility were further characterised by genetic complementation. Sixteen strains were found that carried plasmids with one or more of the known flagellar genes¹². There were four in region I, seven in region II and five in region III. These strains were used to transfer the plasmids into a minicell-producing strain. Minicells were prepared¹³ and purified by multiple sucrose gradients. The minicells were then incubated with ^{35}S -labelled methionine, and after incubation, the total minicell pellet was disassociated, and the peptides were separated by acrylamide gel electrophoresis. Different minicell preparations had different levels of contamination with whole cells. Contamination resulted in a low level of incorporation of label into a large variety of polypeptides. These formed very similar patterns of distribution on the gel, independent of the nature of the plasmid carried by the parent strain (Fig. 2, columns 1 and 2). On the other hand, there were specific, heavily labelled polypeptide

bands that were characteristic of the hybrid plasmid carried by the minicells.

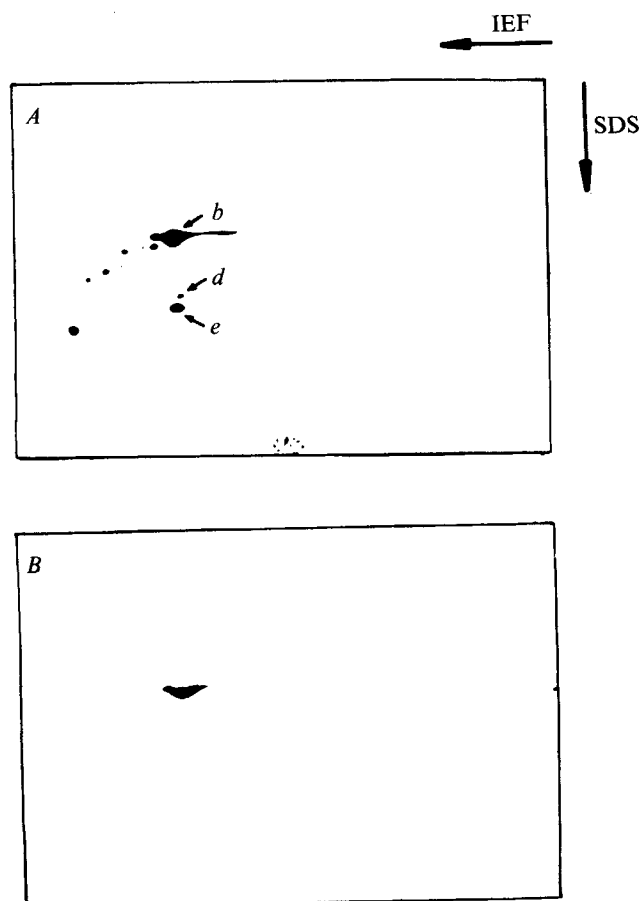
Thus far, minicells from strains that carried a plasmid with *fla* genes mapping in region III (pLC 24-16) were found to synthesise a polypeptide with the same mobility on acrylamide gels as flagellin. Plasmids that carried the *cheB* genes in region II (pLC 21-2 and pLC 24-15) directed the synthesis of polypeptides with the same electrophoretic mobility as the *cheB* gene products synthesised by hybrid *E. coli*- λ bacteriophage (our work, in preparation). Another plasmid carrying the *flaD* gene (pLC 7-18) led to the synthesis of distinctive polypeptides (Fig. 2, column 1) that have not yet been assigned to specific genes. Among the plasmids that have been tested, only those that carried genes in region I (pLC 11-15, pLC 36-11, pLC 24-46 and pLC 35-44) led to the synthesis of a band with the same electrophoretic mobility as the hook subunit (Fig. 2, column 2 and 4). In fact, four of the labelled bands *b*, *c*, *d* and *e* have the same mobilities as polypeptides derived from the purified hook-basal structure complex^{8,14}. These bands correspond to molecular weights of 42,000, 38,000, 30,000 and 27,000.

To determine whether the band with a mobility corresponding to a molecular weight of 42,000 was the same as the hook subunit protein, a minicell extract was pre-

pared and treated with anti-hook subunit antiserum. The precipitate was washed and separated by acrylamide gel electrophoresis. The major radioactive polypeptide found in the precipitate has the same mobility as the hook subunit (Fig. 2, column 3). The same experiment was performed using the two-dimensional isoelectric focusing technique described by O'Farrell¹⁵. Figure 3A shows that there are several different radioactive polypeptides. Spot *b* corresponds precisely with the position of nonradioactive hook subunit protein that was added to the mixture. After precipitation with specific antihook antibody, this spot was the only radioactive polypeptide found on the gel (Fig. 3B). In control experiments with heterologous antibody or with the specific antihook subunit antibody and extracts containing labelled proteins from different minicell preparations, no specific precipitation was found. Finally, in experiments using double diffusion in agar, the antihook subunit formed a specific line of precipitation only with the radioactively labelled material in extracts of minicells carrying the plasmids with genes in region I. Thus, the 42,000 molecular weight polypeptide synthesised in minicells carrying *fla* genes that map in region I behaves like the hook subunit protein.

Some of the other polypeptides that were synthesised in minicells carrying the region I plasmids were similar to other flagellar basal structure components. For example,

Fig. 3 Autoradiograms of two-dimensional gels. Minicell extract for antibody precipitation was prepared as described in legend to Fig. 2 from minicells carrying pLC 11–15. The gel technique was that described by O'Farrell¹⁶. The left side of the gel is the acidic end of the isoelectric focusing run and the right side the basic end. *A*, whole extract before antibody precipitation; *B*, after antibody precipitation.



the 38,000 molecular weight basal structure component had the distinctive property that its mobility on sodium dodecyl sulphate (SDS) gels was affected by the presence of β -mercaptoethanol (β ME). In the presence of β ME, its mobility corresponded to a molecular weight of 38,000 while in the absence of reducing agent, its mobility corresponded to a molecular weight of 36,000 (Fig. 2, column 6 and 5, bands *c* and *c*₁). The polypeptide synthesised in minicells behaved in the same way (Fig. 2, column 7 and 8, bands *c* and *c*₁). Furthermore, the 38,000 molecular weight component of flagellar basal structures is not found on the two-dimensional gels⁶. It is presumably lost during isoelectric focusing. The corresponding band in the minicell preparation also does not appear on two-dimensional gels (Fig. 3A). Finally, polypeptides *d* and *e* (Fig. 2 and Fig. 3A) have mobilities very similar to the basal structure components with molecular weights of 30,000 and 27,000 on two-dimensional gels⁶.

We conclude that a gene that maps in region I specifies the structure of the hook subunit protein. The polypeptide product of the gene carried on the hybrid plasmid was identified by its motility on one- and two-dimensional gels and by its specific precipitation with antihook subunit antiserum. Three other polypeptides with characteristics similar to the basal structure components with molecular weights of 38,000, 30,000 and 27,000 are also synthesised by the plasmid carrying genes in region I. This finding extends the approach previously described involving lambda-*E. coli* hybrids. It is now possible to identify all flagellar gene products by programming their synthesis with cloned fragments of *E. coli* DNA. Initial genetic studies on region I have identified three (*flaK*, *flaL*, *flaM*) flagellar specific complementation groups¹⁶. Further studies are required to define completely the genes in this region and to understand the regulatory mechanisms that control the expression of these genes and coordinate it with the other flagellar genes.

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matters arising

Rock volatility and aerosol composition

GOLDBERG¹ has suggested the possible importance of contributions to the atmospheric burden of trace components from the sublimation of surface minerals. We show here, however, that the observed trace element concentrations could only be achieved by higher temperatures than those encountered on the Earth's surface. Goldberg used a simple kinetic model, where the rate of loss of substance per unit area ϕ from a pure surface under a vacuum is equivalent to the rate at which vapour molecules would reach the surface when the solid is in equilibrium with its vapour² and may be written

$$\phi = p_0 (M/2\pi RT)^{1/2}$$

where p_0 is the equilibrium vapour pressure, M the molecular weight and R , T have their usual meanings. This represents the maximum rate at which molecules can leave the surface. In the presence of other gases, however, the rate will be lower owing to the existence of a boundary layer and transfer of vapour molecules across this layer will be the rate determining step in most cases. The rate of diffusion across a laminar boundary layer from 'Fick's Law' is

$$\phi = \frac{DM}{RT} \frac{\partial p}{\partial x}$$

where D is the diffusion coefficient and x is distance. Trace component concentration in the atmosphere will be low as they are removed rapidly from the bulk atmosphere by convective and chemical processes, $(\partial p/\partial x)$ can be approximated by $p_0/\Delta x$, where Δx is the thickness of the boundary layer (~ 2 mm) (ref. 3). The importance of the diffusion layer can be adequately demonstrated by the example of DDT ($M = 0.354$ kg) where $D = 5 \times 10^{-6}$ m²s⁻¹ and $p_v = 2 \times 10^{-5}$ Pa (ref. 3). The value ϕ arising from a pure DDT surface is calculated as 7.3×10^{-12} kg m⁻²s⁻¹ which is in good agreement with the experimental rates (ref. 3) of 0.8 – 5.5×10^{-12} kg m⁻²s⁻¹, but calculations using the simple kinetic model which neglects the boundary layer suggest rates some four orders of magnitude too high (9.6×10^{-8} kg m⁻²s⁻¹).

When considering components of a mixed solid such as a mineral, the activity (f) of the component must be considered.

Little is known about the activity coefficients applicable to the sublimation of trace components of minerals, but they can be of several orders of magnitude (for example, 10^{-3} to 10^3 , ref. 4). In our model unit activity coefficients were assumed and activity was equated with mole fraction. A solid phase resistance to sublimation must also be considered. When a trace element evaporates from the exposed surface of a matrix, the surface region becomes depleted in the evaporating component. Thus the overall rate of loss may well be controlled by the replenishment of the surface region through transfer of the trace component to the surface from the bulk of the solid. Thus there may be two resistances to transfer, one in the solid phase and one in the gas phase, which limit the evaporative flux. Furthermore, solid phase resistance can also occur in pure solids where structural irregularities which control the sublimation must be regenerated⁵. For the minerals of interest here, the

Table 1 shows the results of calculations using this formula for metals that show anomalously high atmospheric concentrations that cannot be accounted for by crustal or marine inputs. For most of the elements discussed here, vapour pressure data for actual crustal minerals is not available, so that measurements for pure solids as indicated are used for provisional estimates. The final column gives the minimum temperature at which the pressure p^* is achieved. For all the oxides, the calculated temperature is outside the range of validity of the p_0 - T data. The direct extrapolation, however, overestimates the vapour pressure at a given temperature. It is noticeable that even for the volatile elements (As, Se, Sb) the oxides have insufficient volatility to account for their atmospheric levels at ambient temperatures, although temperatures of nearly 70 °C might be achieved at the surface of minerals in tropical regions. For CdO and PbO the temperatures required are well above

Table 1 Properties of some of the elements which behave anomalously in the atmosphere

Element	Atmospheric Concentration (from ref. 6) C (10 ⁻¹² kg m ⁻³)	Weight fraction of the crust (p.p.m.) (from ref. 8)	Mole fraction of crust ($\times 10^3$)†	p^* (10 ⁻⁴ atm)	T (°C)‡
SeO ₂	2	0.05	0.03	4.7	77
As ₂ O ₃	0.5	1.8	0.6	0.03	74
Sb ₂ O ₃	0.3	0.2	0.04	0.18	198
PbO	35	12.5	3.6	0.32	600
CdO	2	0.2	0.1	1.0	680
Hg(1)	5	0.08	0.025	6.6	39

†Using average crustal compositions from ref. 8; the 'average' molecular weight of the crust was computed as 63.5 g mol⁻¹. Weight fractions were then corrected for relative molecular weight.

‡From p_0 - T formulae, in ref. 9.

magnitude of the solid phase resistance is not easily determined and has been omitted. This yields upper limits to the sublimation rate as far as solid phase resistance is concerned.

When sublimation is the sole contribution to the atmospheric burden, the principle of steady state enables ϕ to be equated with the deposition rate per unit area CH/τ , where C is the atmospheric concentration, H is the height of the tropopause (about 13 km) and τ is the tropospheric residence time (about 10 d)¹. Thus the pressure (p^*) needed to account for the observed atmospheric burdens can be calculated from

$$p^* = \frac{CHRT\Delta x}{DMf\tau} \quad (1 \text{ atm} = 10^5 \text{ Pa})$$

ambient, but such temperatures may be achieved in a number of high temperature processes, such as ore roasting or coal burning and in volcanoes. The transfer of the more volatile elements to fly ashes has been noted⁷. As a special case the p^* and T have been calculated for mercury metal, with its high vapour pressure. As can be seen, a temperature of at least 39 °C is needed to account for the observed atmospheric levels if all the crustal burden of mercury existed as the liquid metal. Sublimation of natural mercury ores such as cinnabar (HgS) would, of course be very much slower. Further scrutiny of the p_0 - T data indicates that activity coefficients of at least 10^4 and negligible solid phase resistance are needed to account for the anomalously high atmospheric concentrations of As, Se, Sb, Hg, Pb and

Cd by a sublimation mechanism at ambient temperatures. This is not to imply, however, that such processes could not be important at higher temperatures or lower atmospheric pressures, possibly contributing to the atmospheric chemistry of other planets.

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Control of flight activity in mosquitoes

JONES, in his interpretation of the activity cycle of the mosquito *Culex pipiens fatigans* Wied. in constant light (LL) following a light : dark (LD) regime of 12 h light and 12 h dark¹, stated that it was possible to calculate that the first peak should have occurred about 18 h after light-on or 6 h after the normal time of light-off. In his recordings of activity the main peaks in LD 12:12 were immediately following light-off and light-on. In most field observations of biting activity^{2–4}, in the laboratory cycle of mating activity⁵ and in my laboratory observations of flight activity, the main peaks in the day-length (LD 12:12) typical of the tropics are around midnight and after light-on. By plotting the positions of the peaks in various LD regimes I found the control of flight activity in *C.p. fatigans* to be a combination of light-on some 13–18 h earlier and light-off some 8–14 h earlier (Fig. 1). The mosquito will also respond immediately to light-on in LD 8:16 to LD 20:4. I did not study the activity in LL but in constant dark (DD) following an LD 12:12 I observed a bimodal cycle with one peak following light-on by about 18 h and the other following light-off by about 12 h; these were both repeated twice in DD with a period of about 27 h. Clear double peaks were shown in DD following an LD 4:20 rearing regime; the period in this case was about 24 h. The second peak became much greater with successive cycles to present a result resembling the activity in LL as recorded by Jones¹.

The separate phase-setting effects of light-on (dawn) and light-off (dusk) in a mosquito were first observed for *Aedes*

aegypti L. and formed the basis for suggesting an association between the range of photoperiods in which these separate phase-setting effects reinforce each other and the photoperiod usually encountered by a species throughout its natural range⁶. For dark-active species, such as *Anopheles farauti* Laveran, the optimum photoperiod seems to be determined by the coincidence of a peak entrained by the previous light-off and the peak in response to light-on⁷. *C.p. fatigans*, to judge from the increase in the size of the light-off entrained peak in DD and LL, probably has the same mechanism for determining photoperiod. A theoretical optimum daylength of 12–14 h for *C.p. fatigans*, derived from Fig. 1, compares reasonably well with its known geographical range of 0°–35/40° latitude.

The relevance of the association between photoperiod measurement and the photoperiods within their natural geographical range is not obvious for tropical mosquitoes but increasingly in recent years separate 'dawn' and 'dusk' oscillations have been linked with the

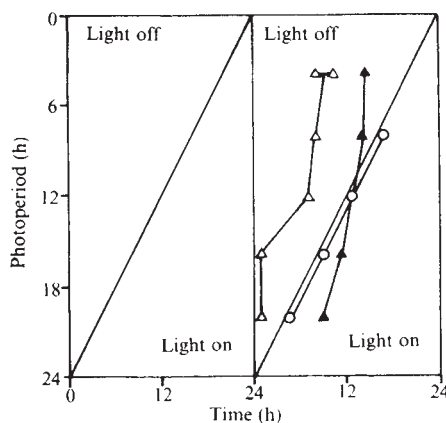


Fig. 1 The positions of the peaks of activity of *C.p. fatigans* relative to light-on and light-off when the mosquitoes are in different light regimes. Diagonal lines divide light-on and light-off periods. Peaks of activity are indicated as: ○, peak at light-on; △, peak following light-on by 13–18 h; ▲, peak following light-off by 8–14 h. Total of 26 individual females was studied; activity was recorded acoustically⁸ at 25 °C; light intensity was 70 lx.

measurement of photoperiod by insects which have a diapause stage in the annual life-cycle⁸. It has not been clearly established, however, whether overt rhythms of activity can be used as indicator rhythms in photoperiodism studies. I have studied several temperate species of mosquito which have at least two generations per year. For *C. pipiens pipiens* L. I obtained graphs of activity peaks which for adults reared in July from larvae collected in southern England (latitude 51 °N, daylength 15 h), that is, a summer generation, gave a theoretical optimum daylength range of 14–17 h,

but for adults taken from hibernation in January, that is, the winter generation, the range was 11–12 h. *C. torrentium* Martini showed a range of 15–18 h for adults reared from larvae collected in mid-July whereas the range for adults reared from larvae collected in mid-September (daylength 13 h) was 12–14 h. The effect of shortening daylengths on summer generation mosquitoes and of lengthening daylengths on winter generation mosquitoes can clearly produce an imbalance between the internal mechanisms controlling flight activity, so there may be a link between overt flight activity rhythms and the photoperiodic mechanism controlling the adult diapause of hibernating mosquitoes.

This work is part of a Ph.D thesis, Brunel University, 1969. I thank the Ministry of Overseas Development and the MRC for financial assistance and Professor J. D. Gillett for his interest and encouragement.

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¹ Jones, M. D. R. *Nature* 261, 491–492 (1976).

² Service, M. W. *Bull. ent. Res.* 54, 601–632 (1963).

³ Teesdale, C. *Bull. ent. Res.* 50, 191–208 (1959).

⁴ Van Someren, E. C. C., Heisch, R. B. & Furlong, M.

Bull. ent. Res. 49, 643–660 (1958).

⁵ Sebastian, A. & de Meillon, B. *Bull. Wld Hlth Org.*

36, 47–52 (1967).

⁶ Taylor, B. & Jones, M. D. R. *J. exp. Biol.* 51, 59–70

(1969).

⁷ Taylor, B. *Nature* 222, 296–297 (1969).

⁸ Saunders, D. S. *J. Insect Physiol.* 20, 77–88 (1974).

JONES REPLIES—Biting and mating depend on flight, but do not necessarily have to show the same pattern; all that is required is sufficient flight activity at the appropriate time. The cycle I have observed in *C.p. fatigans* provides such a permissive framework; it is also consistent with the pattern of flight activity measured in the field using window traps.

Taylor's results^{1,2} could indicate an interesting difference in behaviour between strains (his material originated in Lagos). Confirmation of his results would be useful as the experiments he has quoted were all carried out at the same time using only one batch of insects which had been reared together.

'Dawn' and 'dusk' oscillators may indeed play an important part in the control of mosquito flight activity. This would fit in with the new model for circadian pacemakers proposed by Pittendrigh and Daan³ which they describe as a 'clock for all seasons'.

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¹ Taylor, B. *Nature* 265, 762 (1977).

² Taylor, B. thesis, Brunel Univ. (1969).

³ Pittendrigh, C. S. & Daan, S. *J. comp. Physiol.* 106, 333–355 (1976).

Dirac's continuous creation cosmology and the temperature of the Earth

ROXBURGH¹ has contended that the present form of Dirac cosmology² predicts too low a temperature for the Earth (in the recent past) to be acceptable. He computed the time dependence of the temperature of the Earth with the relation

$$T = \left(\frac{L}{R^2} \right)^{1/4} \quad (1)$$

The solar luminosity $L \sim G^7 \times M_\odot^5 \sim t^3$, when the Dirac prescriptions $G \sim t^{-1}$, $M_\odot \sim t^2$ are used. For the time dependence of the radius of the Earth R in atomic units, Roxburgh proposed and solved the equations of motion ((4) and (4a) in ref. 1). These equations are not valid in atomic units. Being the standard Newtonian equations, they are valid only in Einstein units where, since m is constant, the differentiation between (4) and (4a) in ref. 1 is meaningless. From Roxburgh's equations, we conclude that the orbital radius in Einstein units $\bar{R} = \text{constant}$.

Dirac¹ has argued that with multiplicative creation, $R \sim \bar{R}t$. Equation (1) then yields $T \sim t^{1/4}$, as shown by Canuto and Lodenquai³. Such a time variation cannot be ruled out. While Roxburgh considers Dirac's argument unconvincing, we point out that it is substantiated by dynamic equations of a consistent theory (refs. 4 and 5). In the following, we indicate four equivalent methods by which the correct equation of motion in atomic units can be derived.

(1), With a general transformation of units of the form $d\bar{s} = \beta ds$, where $d\bar{s}$ and ds are the space-time intervals in Einstein and atomic units respectively, the geodesic equation of general relativity transforms into

$$\frac{d}{ds} (\beta v^\mu) + \beta \Gamma_{\nu\lambda}^\mu v^\nu v^\lambda = g^{\mu\nu} \beta_r \quad (2)$$

(2) The gravitational field equations in atomic units given in refs 4 and 5 can be used to derive

$$T_{\mu,\nu} = -3 \frac{\beta_{,\nu}}{\beta} T_{\mu}{}^\nu - \frac{1}{3} T_{\lambda}{}^\lambda \delta_{\mu}{}^\nu \quad (3)$$

Using the stress-energy tensor of a pressureless fluid $T^{\mu\nu} = \rho v^\mu v^\nu$, equation (3) reduces to the generalised geodesic equation (2).

(3) in a gauge covariant theory of gravitation⁴, the tensor equations in general relativity are replaced by co-tensor equations. The geodesic equation is replaced by

$$v^\nu v^\mu{}_{*r} = 0 \quad (4)$$

where v^μ is now a co-vector of power -1 and $*$ indicates co-covariant differentiation. It can be shown that equation (4) is a compact form of equation (2).

(4) Dirac⁵ has given an action principle from which equation (2) can be derived.

With spherical symmetry, the Newtonian limit of equation (2) gives

$$\frac{d}{dt} (\beta r) = -\beta \frac{GM_\odot}{r^2} r \quad (5)$$

This equation admits the angular momentum integral

$$\beta r^2 \dot{\theta} = h = \text{constant} \quad (6)$$

and therefore the reciprocal radius u satisfies the orbit equation

$$\frac{d^2 u}{d\theta^2} + u = \beta^2 \frac{GM}{h^2} \quad (7)$$

Thus, the orbital radius R is given by

$$R \sim \frac{h^2}{GM_\odot \beta^2} \quad (8)$$

In the gauge covariant theory with multiplicative creation as suggested by Dirac, it can be shown that $\beta \sim t^{-1}$. The result $R \sim t$ is thus confirmed.

There is abundant evidence to favour multiplicative creation over additive creation³. The gauge covariant theory is consistent with co-moving multiplicative creation. Roxburgh's suggestion of multiplicative creation at rest does not have a sound theoretical framework and his equation of motion for this case seems to be an *ad hoc* postulate, the consequences of which ought not to be associated with Dirac cosmology.

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¹ Roxburgh, I. W. *Nature*, **261**, 301 (1976).

² Dirac, P. A. M. *Proc. R. Soc. A* **338**, 439 (1974).

³ Canuto, V., & Lodenquai, J. *Astrophys. J.* **211**, 342 (1977).

⁴ Canuto, V., Adams, P. J., Hsieh, S.-H., Tsiang, E. Gauge Covariant theory of Gravitation (NASA preprint, 1976).

⁵ Dirac, P. A. M. *Proc. R. Soc. A* **333**, 403 (1973).

ROXBURGH REPLIES—Canuto *et al.*¹ correctly point out that if the equations of motion in the Dirac² cosmology are Einstein's equations in Einstein units rather than in atomic units as I assumed³, then the orbit of the Earth around the Sun increases in time $R_A \propto t$, so that the temperature on the Earth increases only slowly $T \propto t^{1/4}$. This is only the case if matter is created at rest relative to local

matter, not if it is created at rest relative to the local cosmological rest frame. In this latter case, even if the equation of motion is Einstein's in Einstein units, we have for a system of $N(\tau)$ particles of mass $m(\tau)$

$$\frac{d}{d\tau} (Nm v) + Nm v \frac{1}{N} \frac{dN}{d\tau} = - \frac{GMNm}{r^2_E} \quad (1)$$

and with $Nm = \text{constant}$ in Einstein units this gives

$$\frac{d}{d\tau} (Nv) = - \frac{GMN}{r^2_E} \quad (2)$$

and hence $R_E \propto 1/N^2$. In atomic units $R_A \sim t r_E \propto t/N^2 \propto 1/t^3$ so the equilibrium temperature of the Earth satisfies

$$T_E \propto \left(\frac{L}{R_A} \right)^{1/4} \propto \left(\frac{G^7 M^5}{R_A^2} \right)^{1/4} \propto t^{9/4} \quad (3)$$

which predicts a temperature of about 180 K when the Earth was formed.

The assumption that Einstein's equations are valid in Einstein units, however, does not uniquely predict that the creation rate $\propto t^2$ or that the scale factor in cosmology is $S(t) \propto t$ in atomic units as in the Dirac model. Indeed, if we demand that there be no cosmological constant in Einstein units then one solution is clearly the Einstein de Sitter Universe with $S(t) \propto t^{2/3}$ in Einstein units. This is satisfied with the creation rate such that $N^\circ(\tau) \propto \tau^n$ for any n (my work, to be published). In atomic units this gives a scale factor

$$S(t) \propto t^{\frac{(3n+1)}{(3(n+1))}}$$

For $n = 0$ this is the classical Dirac cosmology $S \sim t^{1/3}$. The equilibrium temperature on the earth for this model then satisfies

$$T_E \propto t^{\frac{(n-5)}{(4(n+1))}} \quad (4)$$

and is constant for $n = 5$. The point is that just to demand that Einstein's equations be valid in units in which mN , G , are constant does not determine the model, a whole family of models are possible that satisfy the large number hypothesis and Einstein's equations in suitable units, only if we demand that in atomic units $S(t) \sim t$, or equivalently that in Einstein units we have a static Einstein Universe, as we reproduce the Dirac model.

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¹ Canuto, V., Adams, P. J., Hsieh, S. H. & Tsiang, E. *Nature* **265**, 763 (1977).

² Dirac, P. A. M. *Proc. R. Soc. A* **338**, 439 (1974).

³ Roxburgh, I. W. *Nature* **261**, 301 (1976).

Devonian palaeogeography of Northern Britain

DONOVAN *et al.*¹ have recently summarised sedimentological data from the Devonian of North Scotland which they believe invalidate my suggestion of a large-scale sinistral shift along the Great Glen Fault (GGF) in late/post Devonian time²⁻⁴. Their main arguments are that "the ORS sediments of the Orcadian Basin on both sides of the GGF are very similar in character and show identical history of development" and that palaeocurrent vectors support sedimentation within a single basin. The authors consider the Orcadian Basin to have been of oval shape, the long axis being of the order of 400 km. If a basin of this size can have similar characteristics throughout there is no reason why the outcrops from a basin only about twice as long (corresponding to my proposed 500-km trans-currence) should not exhibit an equally good match of sedimentological and other features. The evolution of the Orcadian Basin may have been strongly linked with lateral and vertical movements of the GGF, causing simultaneous variation in sedimentary facies, tectonic structures and so on over extensive parts of its length. Furthermore, from the palaeocurrent vectors presented (dominantly from west of the fault) one can devise widely different reconstructions within the framework of a single basin: data from the adjoining shelf areas are very necessary before such information becomes relevant to the problem under consideration.

The available data can equally well be fitted to the model of the Orcadian Basin having subsequently become subdivided by a major transcurrent movement. With a 500-km sinistral displacement the East Shetland Basin, which includes thick basal breccias and coarse fluvial conglomerates derived from a metamorphic/plutonic terrain to the west⁵, would fit in well with the geology of areas around southern Inverness-shire (west of the GGF).

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Statement

IN September 1976 Dr Robert J. Gullis left our laboratory after having spent two postdoctoral years with us. He had been engaged mainly in measuring the levels of cyclic GMP in neuroblastoma cells and neuroblastoma × glioma hybrid cells. We published four papers on this matter together with him. After Dr Gullis had left, several of my colleagues (M. Brandt, J. Traber and D. van Calker) repeated this work, but were unable to reproduce it. Dr Gullis was therefore asked to return to our laboratory and repeat his essential experiments under supervision. During a 2-week period Dr Gullis carried out four series of experiments. After the experimental incubations, the samples were coded. In none of the experiments was Dr Gullis able to obtain his previous results. Neither morphine nor levorphanol nor the enkephalins nor cholinergic agonists changed the level of cyclic GMP in the hybrid cells.

In some of the publications listed below cyclic AMP was determined in the same samples in which cyclic GMP had been determined. The cyclic AMP assays were carried out by other members of the laboratory. But the printouts from the scintillation counter were left to Dr Gullis for evaluation.

Dr Gullis admitted having invented the results of all his experiments. Thus, I should like to let it be known to the scientific community that the following three publications are based on invented data:

Gullis, R. J., Traber, J. & Hamprecht, B. Morphine elevates levels of cyclic GMP in a neuroblastoma × glioma hybrid cell line. *Nature* **256**, 57-59 (1975).

Gullis, R. J., Traber, J., Fischer, K., Buchen, C. & Hamprecht, B. Effects of cholinergic agents and sodium ions on the levels of guanosine and adenosine 3':5'-cyclic monophosphates in neuroblastoma and neuroblastoma × glioma hybrid cells. *FEBS Lett.* **59**, 74-79 (1975).

Gullis, R. J., Buchen, C., Moroder, L., Wünsch, E. & Hamprecht, B. Opiate-like effects of enkephalin on neuroblastoma × glioma hybrids, in *Opiates and endogenous opioid peptides* (ed. Kosterlitz, H. W.) 143-151 (Elsevier, Amsterdam, 1976).

The data on cyclic AMP were falsified by Dr Gullis in a fourth paper (Figs 3 and 4).

Brandt, M., Gullis, R. J., Fischer, K., Buchen, C., Hamprecht, B., Moroder, L. & Wünsch, E. Enkephalin regulates the levels of cyclic nucleotides in neuroblastoma × glioma hybrid cells. *Nature* **262**, 311-313 (1976).

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DR GULLIS WRITES—I wish to disclose the fact that papers published in several journals with myself as principal author are not reliable. The curves and values published are mere figments of my imagination, and during my short research career I published my hypotheses rather than experimentally determined results. The reason was that I was so convinced of my ideas that I simply put them down on paper; it was not because of the tremendous importance of published papers to the career of a scientist.

Therefore I would like to let it be known that the following papers published while I was working in the laboratory of Dr B. Hamprecht are not reliable.

Gullis, R. J., Traber, J. & Hamprecht, B. *Nature* **256**, 57-59 (1975).

Gullis, R. J., Traber, J., Fischer, K., Buchen, C. & Hamprecht, B. *FEBS Lett.* **59**, 74-79 (1975).

Gullis, R. J., Buchen, C., Moroder, L., Wünsch, E. & Hamprecht, B. Opiate-like effects of enkephalin on neuroblastoma × glioma hybrids, in *Opiates and endogenous opioid peptides* (ed. Kosterlitz, H. W.) 143-151 (Elsevier, Amsterdam, 1976).

Another paper in which I was co-author and submitted cyclic GMP values is also wrong in terms of the cyclic GMP content (Figs 3 and 4). The paper is

Brandt, M., Gullis, R. J., Fischer, K., Buchen, C., Hamprecht, B., Moroder, L. & Wünsch, E. *Nature* **262**, 311-313 (1976).

I would also like to disclose the fact that the following papers published with Dr C. E. Rowe are purely hypothesis.

Gullis, R. J. & Rowe, C. E. *Biochem. Soc. Trans.* **1**, 849 (1973); *Biochem. J.* **148**, 197-208; 557-565; 567-581 (1975); *J. Neurochem.* **26**, 1217-1230 (1976); *FEBS Lett.* **67**, 256-259 (1976).

This letter is to point out to the scientific community that the results presented in these papers are wrong and based purely on hypothesis. I must take full responsibility for these unfortunate incidents and have consequently suffered. I hope that my experiences are noted by others, and I would like to apologise to the scientific community and the various people involved.

R. J. GULLIS

¹ Donovan, R. N., Archer, R., Turner, P. and Tarling, D. H., *Nature*, **259**, 550-551 (1976).

² Storetvedt, K. M., *Geol. Mag.*, **111**, 23-30 (1974).

³ Storetvedt, K. M., *Nature*, **249**, 777 (1974).

⁴ Storetvedt, K. M., *Geol. Mag.*, **112**, 94-96 (1975).

⁵ Mykura, W., *Geol. Mag.*, **112**, 91-94 (1975).

reviews

Human qualities and political realities

E. H. S. Burhop

Energy and Conflict: The Life and Times of Edward Teller. By S. A. Blumberg and G. Owens. Pp. xvii+492. (Putnam: New York, 1976.) \$9.95.

THE role of physics in society has been transformed out of all recognition during the past fifty years. The demonstration that at least some of the discoveries of modern physics have immediate relevance for problems of human society has led inevitably to many aspects of physics research becoming inextricably linked with politics. Those physicists who aspire successfully to lead the work in such fields need not only the conventional gifts that make a great scientist—curiosity, creative imagination, enthusiasm, deep insight, critical scepticism and so on—but also a wide understanding of human and social problems and of political realities. This book describes how one such physicist met the challenge.

Edward Teller is one of a remarkable group of Hungarian-born emigrés (including Eugene Wigner, John von Neumann and Leo Szilard) who made great contributions to the development of nuclear energy in the USA. Teller had shown remarkable aptitude for research in theoretical atomic and nuclear physics and in theoretical chemistry, and his original contributions in these fields are very considerable. Unfortunately he was much less successful in dealing with the political problems to which I have referred, with the result that he has become a controversial figure, forfeiting the respect and friendship of many of the physicists whose good opinion he valued most and rightly or wrongly establishing for himself the image of a rather sinister Professor Strangelove figure to large sections of the public.

This biography of Teller traces his career through his early days as a student and brilliant young research physicist in Budapest, Germany and Copenhagen, his emigration to the USA, his involvement with the early work in neutron physics, and nuclear fission; and, throughout World War II and afterwards, his single-minded commitment to the realisation of a thermonuclear weapon, the so called super-bomb. His campaign to secure agreement to a crash program to develop the hydrogen bomb brought him into

conflict with many of his scientific colleagues—especially Robert Oppenheimer, who had been in charge of the development of the wartime fission bomb at Los Alamos. Teller was given the green light to go ahead. In the cold war atmosphere of the time that produced the hysteria of McCarthyism, of which most of Teller's colleagues were thoroughly ashamed, Oppenheimer's opposition to the development of the hydrogen bomb was represented as something sinister, and Eisenhower ordered that Oppenheimer's security clearance should be subject to a hearing, as required by law, to show cause why it should not be rescinded. In the subsequent hearings before a specially constituted commission, Teller's evidence was decisive. The counsel for the Commission cross-examining Teller, put to him the key question "Do you or do you not believe that Dr Oppenheimer is a security risk?" Teller's reply included the damning words "I would like to see the vital interests of this country in hands which I understand better and therefore trust more".

This moment was a watershed in Teller's career. He has never been able to live it down. The Commission went on to remove Oppenheimer's clearance. Oppenheimer continued with his scientific work as Director of the Institute of Advanced Studies at Princeton. But he was a sensitive man and he never fully recovered from the ordeal through which he had had to pass.

Teller was given the opportunity to build up a new weapons laboratory at Livermore, California, in competition with the Los Alamos Laboratory, set up by Oppenheimer during the war, and was able to realise his ambition, which had become almost an obsession, to provide the US Army with its hydrogen bomb.

The story of the development of the hydrogen bomb is a fascinating one and both it, and the monumental clash between the personalities of Oppenheimer and Teller is well told. Both were equals as physicists but it would be hard to imagine two more different personalities. Oppenheimer was a man of great culture with very broad interests and understanding of social problems and their perspectives. Before the War he had identified the true nature of fascism and realised that only by building a popular front with all

forces opposed to it, including the communists, could it be fought and defeated.

Teller was much more limited in his understanding of the broader problems of society. His attitudes were no doubt conditioned by his background and education in Hungary and Germany as the son of a prosperous Jewish lawyer. His family suffered discrimination under the Bela Kun regime of 1919 on account of their bourgeois origins. They witnessed the rise of anti-Semitism in Hungary during the period of the Horthy regime, eventually culminating in the unspeakable excesses against the Jews of the Nazi Arrow Cross Movement. He emerged with a simplistic, uncritical equation of Communism with Nazism and an irrational hatred of the Soviet Union which has undoubtedly coloured his judgment. While Oppenheimer looked beyond the era of the cold war with its "peace by mutual terror" to an era of negotiation, détente and "peace through understanding" which would make the qualitative escalation of nuclear weapons unnecessary, Teller apparently saw and still sees only the need for weapons escalation unlimited.

This book has no doubt been originally conceived as an apologia for Teller, to try to do something to restore his image. Nevertheless it is by no means an uncritical eulogy, and brings out some of the weaknesses as well as the favourable qualities of its subject. It seems to deal objectively with the clash of personalities between Oppenheimer and Teller, the highpoint of the narrative. Teller emerges from the book as a warm and friendly man and, to his credit, with a sincere detestation of secrecy in science. Yet, in the opinion of the reviewer, such desirable personal qualities cannot balance out the great harm his uncompromising hawkish attitudes have done to the prospects of building a peaceful and secure future for mankind. He represents an example and a warning of a very talented scientist with many laudable and human qualities, whose lack of understanding of the social and political forces at work in our society has been a misfortune for himself and his colleagues. □

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Reproduction of eukaryotic cells

Reproduction of Eukaryotic Cells. By D. M. Prescott. Pp. ix + 177. (Academic: New York and London, October, 1976.) \$14.50; £8.85.

ACCORDING to the blurb this book "integrates recent discoveries about the molecular biology of the cell cycle into a comprehensive explanation of cell reproduction". In his preface the author wisely states his purpose more modestly "... to organise into a single source the principal facts and observations on the cell life cycle ... in an effort to increase our overall understanding of how these cells reproduce themselves and how this reproduction is regulated". The book interprets eukaryote cells to be mainly vertebrate derived, but we do get occasional forays into yeasts and amoeba. The monograph is intended to be (and indeed is) complementary to Mitchison's book, *Biology of the Cell Cycle* (Cambridge University: Cambridge and London, 1971), which dealt extensively with microorganisms.

After briefly introducing the cell cycle and discussing the pattern of cell mass increase between successive divisions the author plunges straight into cell synchronisation techniques. For many workers, cell synchrony is a prerequisite for any successful biochemical or molecular biological study of cell cycle control. The author does not make the usual mistake of ignoring the variability of G_1 but he seems puzzled by the failure of many attempts to achieve perfect cell synchrony. In spite of the 560 references (mostly we are told published since 1971), no mention is made of models of the cell cycle which not only predict that G_1 must of necessity be variable, but put this variation on a quantitative footing (Burns and Tannock, *Cell Tissue Kinet.*, **3**, 321, 1970; Smith and Martin, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1263, 1973). Whether these explanations of the variation of G_1 are accepted or not the variability of G_1 is an undeniable and unavoidable fact. As a consequence, temporal studies on the biochemistry of cell division are fraught with pitfalls. For instance, even within a mitotically synchronised population some cells enter S-phase within a few hours but other cells do not start DNA synthesis until many hours later. As a result, biochemical phenomena observed in an "early G_1 " population are a mixture of events occurring in some cells just before DNA synthesis begins and events that occur in cells that will

sit around for many hours before initiating S-phase. In "mid G_1 " or "late G_1 " the situation is worse. This unavoidable problem means that much of the biochemistry of G_1 is at best of questionable value and at worst useless. Synchronisation at other stages of the cell cycle is slightly more successful, but it is G_1 that is at the centre of cell proliferation control. A knowledge of what happens in G_1 will only come with a proper understanding of its variable nature and the nature of its variation. Until then even the best of monographs can throw little light on the subject.

Although those who have not been exposed to the cell cycle literature will find much of this book of interest, it is a pity that the author has drawn his brief so narrowly. The "molecular biology" we are promised on the dust-jacket turns out to be a rather dry

series of studies with inhibitions of protein and RNA synthesis. There is nothing (for instance) on the role of microtubules in the formation of the mitotic spindle, little on the process of cytokinesis and very little (apart from a discussion of RNA and protein synthesis) about the process of mitosis itself. There is a chapter on that slumping commodity cyclic AMP but little indication of what interesting futures the author would invest in.

In short, although the book is a useful source of references about the bare essentials of the cell cycle it fails to convey much of the excitement which surrounds the study of cell division and its control. **Robert Shields**

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Characteristic seeds

The Seeds of Dicotyledons. By E. J. H. Corner. Vol. 1: pp. ix + 311; Vol. 2: pp. 552 (illustrations.) (Cambridge University: Cambridge, London and New York, 1976.) Vol. 1: £15; Vol. 2: £25.

THE flowering-plant taxonomist, struggling with a welter of data, may at times be forgiven for regarding Occam's razor as a more useful tool than the microtome. Not so his brother the phylogenist, for Nature has concealed the origins and ancestry of the angiosperms with such skill, that every scrap of evidence may be required to unravel the mystery, and transform hypothesis into proven fact. In this respect, seeds (surely of some significance in seed-plants) have been seriously underrated by taxonomists and phylogenists alike, perhaps because they are not the easiest things in the world to examine, or to describe.

Professor Corner claims that every natural family of dicotyledons has a characteristic seed, and that, in consequence, the classification of families into orders should be consistent with their seeds. To substantiate this claim 218 pages of volume 1 of *The Seeds of Dicotyledons* consist of brief (but perfectly ample) descriptions of seeds and ancillary structures from most dicotyledonous families, together with descriptions of the seeds of many genera within these families. Volume 2 furnishes the necessary illustrations, 647 plates in all, comprising what must amount to several thousand individual figures, each a clear *camera lucida* outline, the salient features emphasised by

judicious stippling and shading. The amount of work involved in sectioning (free-hand) so much material at every stage of development from unfertilised ovule to ripe seed, and in illustrating the resultant observations, must have been stupendous, so that it is almost mean to suggest that the plates might have been even more useful had the individual figures and corresponding legends been more uniformly labelled with numbers or letters.

The phylogenetic conclusions which Professor Corner reaches as a result of his seed studies are (coming from the father of Durianology) not quite so startling as might have been supposed. All depends upon one's criteria of primitiveness. Professor Corner regards the elaborations of the Myristicaceae seed as the closest approximation to the primitive; thereafter it is but a step to the *Magnoliaceae*, *Annonaceae*, *Lauraceae* and *Monimiaceae*, and indeed to a situation which, through Hutchinson, Takhtajan and others, has become so familiar that we may well be too inclined to take it for granted. If so, the hypothesis that *Paeoniaceae*, *Cucurbitaceae* and *Convolvulaceae* belong to the same phyletic line; that *Grossulariaceae* should be allied to *Cruciferae* and *Polygalaceae*, and *Proteaceae* to *Papaveraceae* and *Fumariaceae* will jolt us out of complacency, and perhaps make us question (as Professor Corner anticipates) if "agreement in seed-structure should be employed to align such diverse plants in a phyletic series". I take his word for it that "it can stand enquiry". It is nonetheless surprising. **R. D. Meikle**

R. D. Meikle is carrying out research at the Herbarium, Royal Botanical Gardens, Kew, Surrey, UK.

Theory and practice of micrometeorology

Vegetation and the Atmosphere. Edited by J. L. Monteith. Vol. 1: Principles. Pp. xx+287. £10. Vol. 2: Case Studies. Pp. xix+439. £15. (Academic: London and New York, June 1976.)

This book attempts to give an up-to-the-minute account of the theory (volume one) and practice (volume two) of micrometeorology with special emphasis on its application to problems in plant ecology. On the whole it succeeds admirably.

I was disappointed with volume one. Although all the articles were individually very good, there was a lack of correlation between them. If the purpose of the introductory chapter, (Micrometeorology and ecology) was to encourage ecologists to use micrometeorology, then it would confuse rather than enlighten. It is unrealistic to expect the interested reader to appreciate the sections by Ross (Radiative transfer and plant communities) and Thom (Momentum mass and heat exchange of plant communities) without a general introduction on micro-

meteorology. Ross in the opening paragraphs suggests that up to 28% of total solar radiation may be stored in photosynthetic products. Are Russian plants more efficient?

Chamberlain's article (Movement of particles in plant communities) is interesting but doesn't fit in with the general theme; I also felt there was a need for another chapter on "What, where and how many measurements should be made", before Szeicz's paper on instruments.

Volume two should find a place on the bookshelves of all micrometeorologists, crop physiologists and ecologists. It is a very useful collection of articles on the micrometeorology of various crops; temperate cereals, maize, rice, sugar beet, potatoes, sunflower, cotton, pasture grass, and citrus orchards; and natural communities: coniferous, deciduous and tropical forests, swamp, grassland and tundra. Although the treatment varies from author to author, the editor has imposed a common set of symbols, which is of great assistance to the reader. The contributors to the second volume have, in many cases, included their own theory sections, although this has been adequately covered in the first volume (even a table is duplicated; p39 and

p41, respectively). I would recommend the newcomer to the subject to read the second volume first. He will gain a basic understanding of the subject, its limitations and its applicability; he would then be prepared for the detailed theory of volume one.

The tabular index of volume two is useful, but as it requires a good knowledge of the subject it should have been complemented by a general index. The index in volume one is not comprehensive—for example, Momentum flux, which merits a chapter sub-heading, is omitted; and Respiration, which occurs several times, is only listed once.

Despite its faults, *Vegetation and the Atmosphere* is a most useful contribution to the subject. It is a landmark in micrometeorology for the methods to have become well enough established and sufficiently used to justify a work of this size.

Professor Monteith is to be congratulated both for editing the volumes and introducing the common symbols table which could usefully be adopted by all other workers in this subject.

K. J. Parkinson

K. J. Parkinson is Senior Scientific Officer in the Department of Physics at Rothamsted Experimental Station, Harpenden, UK.

Chalones not proven

Chalones. Edited by John C. Houck. Pp. xiii+510. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1976.) Dfl. 165; \$63.50.

CHALONES have been proposed as diffusible messenger molecules responsible for a negative feedback mechanism controlling cell division. They are, it is claimed, tissue specific but not species specific. Experiments designed to show that tissue extracts contain such inhibitors of cell division have provided controversy for over a decade, principally because of the technical problems associated with the assay of partially purified crude tissue extracts. The preparations themselves must not be inherently cytotoxic, nor contaminated with bacterial products producing cytotoxic or cystostatic effects. *In vitro* assays based on the incorporation of radiolabelled thymidine, although they can measure decreases in mitosis, could also indicate alterations in thymidine metabolism unrelated to cell replication. As a further complication, *in vivo* studies could involve the immunological ramifications of injecting foreign substances.

The majority of the chapters in this book, including several by the editor and his colleagues, are reviews of the current state of research on chalones

in particular tissues. In some of these, but not all, the assay problems outlined above have been carefully considered. The remaining chapters contain more general information, and cover such topics as the control of cell division, the assay of proliferation inhibitors and the history of chalones, this last a comprehensive and entertaining review by O. H. Iverson. *Chalones* is therefore a *pot-pourri* both in the selection of contents and the views expressed, which range from the speculative and optimistic to the factual and cautious. Although the workers whose views are recorded in the book cannot be faulted for their lack of enthusiasm, they, like many others working on tissue extracts, have still failed to come to grips with the formidable problems of biochemical isolation. As the editor points out in his introductory chapter, in 1970 "most biomedical scientists felt that if a name were granted to a functional material, then that material should be . . . isolated, reasonably purified, and certainly characterised as far as to its composition". This book records the points in the case for chalones. There is some progress, but in the circumstances the only judgement yet possible is "not proven". A. S. Hamblin

Anne Hamblin is a member of the scientific staff at the Kennedy Institute of Rheumatology, London, UK.

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Iron meteorites

Handbook of Iron Meteorites: Their History, Distribution, Composition and Structure. By V. F. Buchwald. Vols 1-3 + Suppl. Vol. 1: pp.xii+1-244+8; vol. 2 pp.245-820+8; vol. 3: pp.821-1,418+8. (University of California: Berkeley, Los Angeles and London, 1976.) £98.

THIS HANDBOOK weighs 6.39 kg, or somewhat less than "the most common size of an iron meteorite" (p27). It is a unique monograph in three volumes, and a fitting tribute to the author as scientist-explorer and to his patience and determination in tracking down meteorites from the deserts of Greenland to the museums of Mexico.

Volume 1 is a general introduction under various headings such as "The minor bodies of the solar system" and "Statistical and historical notes". "Classification" includes stones and stony-irons in addition to irons; that for the C3-C4 carbonaceous chondrites was never really accepted and was superseded by 1972, when the book was largely completed; the author lists Shergotty as an "anomalous achondrite", but omits Padvarninkai and Zagami (*Nature* 213, 1, 111; 1967). The modern structural and chemical classification of iron meteorites is lucidly outlined and well illustrated using figures and tables, and the mineralogy of irons and their primary (cooling) and secondary (for example, shock-produced) structures are comprehensively described.

The text of the first volume ends with "Meteorite ages", which is too brief a summary and sometimes misleading. It is stated, for example, that the "majority" of meteorites dated by the Rb-Sr method lie on the same 4.55 Gyr isochron "and thus are cogenetic". I think that few scientists, if any, would agree that Estherville, Nakhla, Orgueil, Weekeroo Station and Forest City (also Apollo 12013!) are cogenetic (Fig. 229A). After the text are various appendices summarising data on different aspects of meteorites, together with a wide list of references not quite comprehensive to the end of 1972. In this introductory volume, I would like to have seen a glossary of terms for the benefit of the wide range of scientists to whom this book should be of interest—for example, what is a Hugoniot curve? (p135).

Volumes 2 and 3 are the forte of the work. In these are given, in alphabetical order, detailed and excellently illustrated accounts of the iron meteorites. Such information is *not* available elsewhere. When possible, a history of

the meteorite is presented thoughtfully and sensitively—for interesting reading see, for example, "Ainsworth" and its probable relatives, and "Elbogen". Then comes the location of material, followed by the chemical and structural 'fingerprint' of the iron, if known. The author, with J. T. Wasson and co-workers, by establishing the identity of various irons has rendered a great service to meteoriticists, and this is apparent from the book. Not all diagnoses, however, are apparently proving to be correct; "Verkhne Dnieprovsk" not only covers mis-labelled Augustinovka but includes the British Museum (Natural History) specimen which belongs to Group IIE and probably represents a distinct iron (see J. T. Wasson, *Meteorites*, Springer, Berlin, 1974); new and as yet unpublished measurements of the distribution of Ni between the phosphide and metal indicate that Buchwald's "North Chile" might include two different meteorites.

Spokes missing in ecological wheel

Ecological Relationships. By N. Gilbert, A. P. Gutierrez, B. D. Frazer and R. E. Jones. Pp. 156. (Freeman: Reading and San Francisco, December 1976.) £3.50.

THIS book is very different from other recent ecological texts. It advocates that the key to understanding ecological relationships is to build detailed computer simulations; these should be based on a comprehensive knowledge of the behaviour, physiology and population characteristics of the component organisms, studied in the field, where the effects of climate and season play an overwhelming role. Simple models, laboratory experiments and general abstractions are to be avoided. It is a point of view which obviously deserves to be aired, but it is not this, but rather its faults, which sets the book apart.

It is full of minor irritations. The title is misleading; it is not about ecological relationships in general, but mainly about the authors' own work on aphids, ladybirds and cabbage-white butterflies. A large number of key references are in that murky area from "unpublished" to "in press", and since many of the methods and results are presented in a most cursory manner, their inaccessibility is frustrating. On the other hand, to occupy approximately 10% of the text with routine Fortran smacks of self-indulgence, particularly when one of the few really interesting results in the book (p30), dealing with the maximisation of fitness in aphids, is presented without any

But this must not detract from the real value of the work as a source of data, for although interpretation may change (as the author admits), the data should not, except for additions.

This book is a necessity for reference use by all concerned with meteorites, and in addition should be of interest to metallurgists, geochemists and other earth scientists. And apart from that, it makes pleasant browsing, reading the varied histories of these unusual and often revered objects. Although the price is high, there are 21.25 illustrations per £, there being 2,124 figures; the price therefore seems reasonable. Any European wishing to save should try to have the book bought in the USA and mailed to Europe.

Robert Hutchison

Robert Hutchison is a Principal Scientific Officer in the British Museum (Natural History), where he is responsible for the national collection of meteorites.

of the "fairly involved algebra" used to derive it.

I have other, more serious criticisms. The authors dismiss "simplistic theories" without apparently taking the trouble to understand them. Thus, having rejected the concepts of population equilibria and stability, they nevertheless find it necessary to talk about "stability" on p75, but put it in quotation marks and qualify it by saying "however defined", thus ensuring that it is meaningless. They attack predator-prey models but re-discover most of the things that current theory already incorporates, such as the voracity and activity of the predators (p80), their response to patchily distributed prey (p49), the importance of limited searching time (p39 and p135) and time wasted in non-hunting activity (p41). And on p81, they re-discover the properties of *r* and *K* species, cursorily dismissed earlier in the book, instead labelling them "ephemeral" and "sessile". I could give many more examples.

Who is this book aimed at? Certainly, it isn't suitable for undergraduates, because although it advocates a methodology, its treatment and use of the literature is too superficial to teach them much. It might be useful to research workers, but it is more likely to put them off than convert them. There is obviously an urgent need to test ecological ideas in the field, but in saying so, this book seems merely to have rediscovered the wheel: unfortunately it has a lot of spokes missing and its square.

John Lawton

John Lawton is Lecturer in Ecology at the University of York, UK.

announcements

Appointments

Professor G. E. Fogg, as President of the Institute of Biology.

Dr E. F. Henzell, as Chief of the CSIRO Division of Tropical Crops and Pastures, Brisbane.

Awards

Gordon A. Riley is winner of the annual AAAS-Rosenstiel Award in Oceanographic Science. The prize of \$5,000 was awarded to Dr Riley for pioneering the concept of the ocean as a biological system interacting with physics and chemistry.

Meetings

9–10 March, **6th Technical Seminar**, Manchester (J. Dunmore, National Society for Clean Air, 136 North Street, Brighton, UK).

10–11 March, **Telecommunications in the 1980s and After**, London (P. B. Carter, The Royal Society, 6 Carlton House Terrace, London SW1).

16–17 March, **The Terrestrial Ecology of Aldabra**, London (P. B. Carter, The Royal Society).

16 March, **Surface and Sub-surface Analytical Techniques**, London (The Institute of Physics, 47 Belgrave Square, London SW1).

22–24 March, **Annual Ocean Thermal Energy Conversion Conference**, New Orleans (D. F. Myer, OTEC Conference, University of New Orleans, Louisiana 70122).

31 March–2 April, **Industrial Drug Discovery—Factors Governing its Effectiveness**, York (R. W. Brimblecombe, Smith, Kline & French Laboratories, Welwyn Garden City, UK).

31 March–1 April, **PAG Symposium on Safety of Single Cell Protein for Animal and Human Feeding**, Milan (S. Garattini, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea, 62-20157 Milano, Italy).

12–16 April, **Development and Differentiation in the Cellular Slime Moulds**, Porto Conte, Sardinia (J. M. Ashworth, University of Essex, UK).

28 April, **Electronics in Transport**, London (Institute of Physics, 47 Belgrave Square, London SW1).

8–12 May, **68th Annual Spring Meeting**, New York (American Oil Chemists Society, 508 South 6th Street, Champaign, Illinois 61820).

5–9 June, **Nutritional Factors in Differentiation / Mechanisms of Cellular Control**, New Orleans (F. H. Kasten, Louisiana State University Medical Center, 1100 Florida Avenue, New Orleans, La 70119).

27 June–1 July, **Enzymes and their Use in Analysis and Clinical Diagnosis**, Cambridge Mass. (Director, Room E19-356, MIT, Cambridge, Mass. 02139).

30 June, **Laser Processing of Engineering Materials**, Slough, UK (Fulmer Research Institute Ltd / UKAEA, J. H. C. Maple, UKAEA Culham Laboratory, Abingdon, Oxon, UK).

9–14 July, **10th International Congress on Sedimentology**, Jerusalem (G. Gvirtzman, Israel Geological Survey, 30 Malkhei Street, Jerusalem 95501, Israel).

3–5 August, **26th Annual Conference on Applications of X-ray Analysis**, Denver (M. Cain, Denver Research Institute, University of Denver, Colorado 80208).

Person to Person

Oil aerosols may be a potential safety hazard and R. D. Athey remembers a newspaper report of pneumonia induced by a medicinal vaporiser. Readers recalling fuller details of this report, or with information on the constitution of oil aerosols likely to produce pneumonia are asked to contact R. D. Athey, 3122 Athens Road, Silver Lake Village, Ohio 44224.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

21–27 August, **5th International Conference on Birth Defects**, Montreal (The Secretariat, c/o Holland Organising Centre, 16, Lange Voorhout, The Hague, the Netherlands).

21 August–3 September, **Molecular Physics of Liquid Crystals**, Cambridge, UK (G. R. Luckhurst, Chemistry, The University, Southampton, UK).

5–9 September, **Immunity in Parasitic Diseases: Antigens and Immune Mechanisms**, Grignon-Yvelines, France (Secretary, Laboratoire d'Immunologie INRA F-78850, Thiverval-Grignon, France).

11–16 September, **11th World Congress of Neurology**, Amsterdam (The Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, The Netherlands).

4–7 October, **Hormones and Cell Regulation**, Bishenberg, France (J. E. Dumont, Bishenberg-IRIBHN-Faculté de Médecine 20, rue Evers B-1000, Brussels, Belgium).

10–13 October, **25th International Meeting on Transportation and Communications**, Genoa (International Institute of Communication, Villa Piaggio, Via Pertinace, 16125 Genova, Italy).

March 7–10, 1978, **Maritime and Aeronautical Satellite Communication and Navigation**, London (Institution of Electrical Engineers, Savoy Place, London, WC2).

April 10–12, 1978, **Private Electronic Switching Systems**, London (Institution of Electrical Engineers, Savoy Place, London, WC2).

23–28 April 1978, **3rd International Symposium on the Natural Radiation Environment**, Houston (T. F. Gesell, University of Texas Health Science Center at Houston, PO Box 20186 Houston, Texas 77025).

May 6–12, 1978, **11th Commonwealth Mining and Metallurgy Congress**, Hong Kong (M. J. Jones, Institution of Mining and Metallurgy, 44 Portland Place, London, W1).

Reports and Publications

Other countries—December

Prescription Drug Industry—Factbook '76. Pp. iii + 86. (Washington, DC: Pharmaceutical Manufacturers Association, 1155 Fifteenth Street, 1976.) [1712]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15385: *Bacillus thuringiensis*: Its Effect on Environmental Quality. Prepared by C. W. Forsberg, with contributions by Margaret Henderson, Ellen Henry and J. R. Roberts. Pp. 134. (Ottawa: Publications, NRCC/CNRC, 1976.) \$2. [1712]

CERN: European Organization for Nuclear Research. CERN 76-19: Geodesy and Metrology at CERN—A Source of Economy for the SPS Programme. By J. Gervaise. Pp. v + 19. (Geneva: CERN, 1976.) [1712]

Fisheries Research Board of Canada. Annual Report 1975. Pp. 20. (Ottawa: Environment Canada, Fisheries and Marine Service, 1976.) [1712]

Education in Nutrition for Physicians and Dentists: An Annotated Bibliography. Pp. xx + 127. (Washington, DC: The Nutrition Foundation, 888 Seventeenth Street, NW, 1976.) \$2. [1712]

Magnetocrystalline and Uniaxial Anisotropy in Epitaxial (110) Ni and Ni-Fe Thin Films. Edited by G. Matsumoto. Pp. 87. (Sapporo, Japan: Research Institute of Applied Electricity, Hokkaido University, 1976.) [1712]

Smithsonian Contributions to Zoology, No. 240: On the Troglolithic Shrimps of the Yucatan Peninsula, Mexico (Decapoda: Atysidae and Palaemonidae). By H. H. Hobbs, III, and Horton H. Hobbs, Jr. Pp. iii + 20. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [1712]

Consensus Among Experts: The Unholy Grail. By Louis Lasagna. Pp. 12. (Rochester, New York: The Center for the Study of Drug Development, 1976.) [1712]

National Research Council Canada. Annual Report on Scholarships and Grants in Aid of Research, 1975/1976. Pp. 582. (Ottawa: National Research Council of Canada, 1976. \$2.50.) [1712]

- A Guide to Bird-Watching in Denmark. By Jeremy Sanders and Karin Berg. Pp. 63. (Stockholm: AB Grafisk Formgivning, J. Sanders, AB: Hitchin, Herts: J. Tidy, 9 Freewaters Close, Ickleford, 1976.) £1.80 [2012]
- Smithsonian Contributions to Paleobiology. No. 24: Permian Brachiopods of West Texas. V. By G. Arthur Cooper and Richard E. Grant. Pp. vi + 2609-3159. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [2012]
- US Department of the Interior: Geological Survey. Professional Paper 813-H: Summary Appraisals of the Nation's Ground-Water Resources—Arkansas-White-Red Region. By M. S. Bedinger and R. T. Sniegocki. Pp. iv + 31. Professional Paper 907-D: Fluid-Inclusion Petrology—Data from Porphyry Copper Deposits and Applications to Exploration. By J. Thomas Nash. Pp. vi + 16. Professional Paper 974: Type Sections and Stratigraphy of the Members of the Blackleaf and Marias River Formations (Cretaceous) of the Sweetgrass Arch, Montana. By W. A. Cobban, C. E. Erdmann, R. W. Lemke and E. K. Maughan. Pp. iii + 66. (Washington, DC: US Government Printing Office, 1976.) [2012]
- Australia: Commonwealth Scientific and Industrial Research Organization. CSIRO Twenty-eighth Annual Report, 1975/1976. Pp. 75. (East Melbourne: CSIRO, 1976.) [2112]
- Socioeconomic Impact of Earthquake Prediction on Government, Business, and Community: Research Findings, Issues and Implications for Organizational Policy. By J. Eugene Haas and Dennis S. Mileti. Pp. 39. (Boulder, Colorado: Institute of Behavioural Science, University of Colorado, 1976.) [2112]
- Science Council of Canada. Background Study No. 38: Human Goals and Science Policy. By R. W. Jackson. Pp. 134. (Ottawa: Printing and Publishing, Supply and Services Canada, 1976.) \$4.80. [2112]
- US Department of the Interior: Geological Survey. Professional Paper 919: Volcanic Suites and Related Caudrons of Timber Mountain-Oasis Valley Caldera Complex, Southern Nevada. By F. M. Byers, Jr., W. J. Carr, Paul P. Orkild, W. D. Quinlivan and K. A. Sargent. Pp. ix + 70. (Washington, DC: US Government Printing Office, 1976.) [2112]
- The Philippine Journal of Coconut Studies. Vol. 1, No. 1, September 1976. Published quarterly. Pp. 1-46. (Ermita, Manila: Philippine Coconut Research and Development Foundation, Inc., 1976.) [2112]
- Smithsonian Contributions to Zoology. No. 237: Shallow Water Procraian Crabs from the Pacific Coast of Panama and Adjacent Caribbean Waters (Crustacea: Anomura: Porcellanidae). By Robert H. Gore and Lawrence G. Abele. Pp. iv + 30. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [2112]
- Flore des Mascareignes: La Réunion, Maurice, Rodrigues. Comité de Rédaction: J. Bosser, Th. Cadet, H. R. Julien et W. Marais. 110. Goodeniaceae, 111. Campanulaceae. (Mauritius: Sugar Industry Research Institute; Kew: Royal Botanic Gardens; Paris: l'Office de la Recherche Scientifique et Technique Outremer, 1976.) [2112]
- Australian Academy of Science. Report No. 30: Report of a Committee on the Problem of Noise. Pp. 101. Report No. 21: Report of a Committee of Climatic Change. Pp. 92. (Canberra City: Australian Academy of Science, 1976.) [2112]
- Annals of the South African Museum. Vol. 72, Part 3: *Notosuchus*—The Oldest Known Rhynchosaur. By Robert L. Carroll. Pp. 37-57. R.2.50. Vol. 72, Part 4: *Galesphyrus capensis*, a Younginid Eosuchian from the Cistecephalus Zone of South Africa. By Robert L. Carroll. Pp. 59-68. R.2. (Cape Town: South African Museum, 1976.) [2112]
- Can Desert Encroachment Be Stopped?—a Study with Emphasis on Africa. Edited by A. Rapp, H. M. Le Houérou and B. Lundholm. (Ecological Bulletin No. 24.) Pp. 241. (Stockholm: Editorial Service of NFR, Box 23136, 1976.) [2112]
- United States Department of the Interior: Geological Survey. Water-Supply Paper 2038: Hydrology and Environmental Aspects of Erie Canal (1817-99). By W. B. Langbein. Pp. vi + 92. (Washington, DC: US Government Printing Office, 1976.) [2112]
- National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15023: NTS (Nitrilotriacetic Acid)—an Ecological Appraisal. By A. Prakash. Pp. 46. (Ottawa: Publications, NRCC/CNRC, 1976.) \$1.25. [2112]
- India Meteorological Department. Meteorological Monograph—Synoptic Meteorology No. 1/1976: Southwest Monsoon. By Y. P. Rao. Pp. 367. (Civil Lines, New Delhi: The Controller of Publications, 1976.) Rs.84; £9.80; \$30.24. [3012]
- Indian Forest Records (New Series). Management and Mensuration. Vol. 1, No. 1: Crop Volume Tables for *Eucalyptus Grandis*. By G. C. Pande and R. C. Jain. Pp. 16. Rs.1.25; 15p; 45 cents. Utilization. Vol. 5, No. 1: Indian Forest and Forest Products Terminology. Part 2: Utilization. (Forest Products Research Extension, Utilization and Trade). Compiled by the Editorial Board, Forest Research Institute and College, Dehra Dun. Pp. 198. Rs.4.90; 58p; \$1.77. (Delhi: Controller of Publications, 1975 and 1976.) [3012]
- Norsk Polarinstittutt. Meddelelser. No. 106: Utforskningen av Kontinentalskollene i Barentshavet—fra Fridtjof Nansens tid til i Dag. Av Tore Gjelsvik. Pp.42. (With English Summary). (Oslo: Norsk Polarinstittutt, 1976.) [3112]
- Science Council of Canada. Study on Population, Technology and Resources. Food Production in the Canadian Environment. By Barbara J. Geno and Larry M. Geno. (Perceptions 3). Pp. ix + 71. (Ottawa: Printing and Publishing, Supply and Services Canada, 1976.) \$2.80. [3112]

UK & Ireland—January

- Health and Security: Report on the Public Provision for Medical Care in Great Britain. By Max Gammom. Pp. 48. (London: St. Michael's Organisation, 80 Highgate West Hill, N6, 1976.) £4; \$8. [51]
- The International Vegetarian Health Food Handbook, 1977/78. Edited by Joanna Lawton. Pp. 232. (Altrincham, Cheshire: The Vegetarian Society of the United Kingdom, Parkdale, Dunham Road, 1976.) 90p. [61]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 284. No. 1318: The Influence of High Pore-Water Pressure on the Strength of Cohesionless Soils. By A. W. Bishop and A. E. Skinner. Pp. 91-130. UK £2.60; Overseas £2.70. No. 1319: Oxygen Isotope Abundance Measurement in Fine Size Fractions of Luna 16 and 20 Soil. By R. D. Beckinsale, S. H. U. Bowie, and J. J. Durham. The Separation and Sub-division of Two 0.5 g Samples of Lunar Soil Collected by the Luna 16 and 20 Missions. By C. T. Pillinger and A. P. Goward. Carbon Chemistry of the Luna 16 and 20 Samples. By C. T. Pillinger, G. Eglinton, A. P. Goward and A. J. T. Jull. Magnetic Characteristics of Luna 16 and 20 Samples. By A. Stephenson, D. W. Collinson and S. K. Runcorn. Mössbauer Studies of Luna 61 and 20 Lunar Soils. By T. C. Gibb, R. Greatrex and N. N. Greenwood. ⁴⁰Ar-³⁹Ar Dating of Luna 16 and 20 Samples. By P. H. Cadogan and G. Turner. Pp. 131-177. UK £2.90; Overseas £3. No. 1320: The Breakdown of Superfluidity in Liquid ⁴He—An Experimental Test of Landau's Theory. By D. R. Allum, P. V. E. McClintock, A. Phillips and R. M. Bowley. Pp. 179-224. UK £2.75; Overseas £2.85. (London: The Royal Society, 1977.) [61]
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